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pRL4

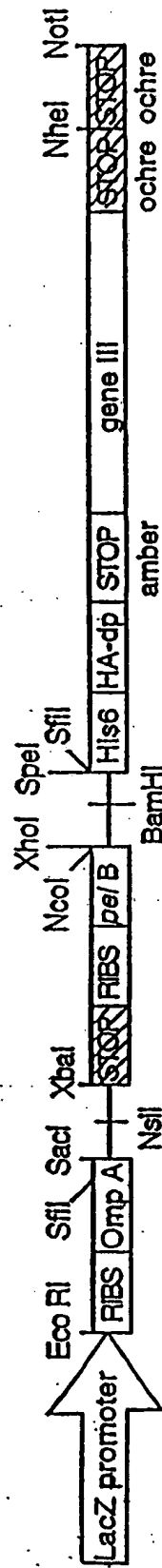


Fig. 1

Human Antibody sequence (TT sequence) (SEQ ID NO: 54)

Heavy Chain: cloning sites Xho I and Spe I are underlined

```
1      11
gag gtg cag ctg CTC GAG CAG TCT GGG GCT GAG GTG AAG AAG CCT GGG TCC TCG GTG AAG
glu val gln leu leu glu gln ser gly ala glu val lys lys pro gly ser ser val lys
21      31
GTC TCC TCC AAG GCT TCT GGA GGC ACC TTC AAC AAT TAT GCC ATC AGC TGG GTG CGA CAG
val ser cys arg ala ser gly gly thr phe asn asn tyr ala ile ser trp val arg gln
41      51
GCC CCT GGA CAA GGG CTT GAG TGG ATG GGA GGG ATC TTC CCT TTC CGT AAT ACA GCA AAG
ala pro gly gln gly leu glu trp met gly gly ile phe pro phe arg asn thr ala lys
61      71
TAC GCA CAA CAC TTC CAG GGC AGA GTC ACC ATT ACC GCG GAC GAA TCC ACG GGC ACA GGC
tyr ala gln his phe gln gly arg val thr ile thr ala asp glu ser thr gly thr ala
81      91
TAC ATG GAG CTG ACG AGC CTG AGA TCT GAG GAC ACG GCC ATA TAT TAT TGT GCG AGA GCG
tyr met glu leu ser ser leu arg ser glu asp thr ala ile tyr tyr cys ala arg gly
101     111
GAT ACG ATT TTT GGA GTG ACC ATG GGA TAC TAC GCT ATG GAC GTC TGG GGC CAA GGG ACC
asp thr ile phe gly val thr met gly tyr tyr ala met asp val trp gly gln gly thr
121     131
ACG GTC ACC GTC TCC GCA GCC TCC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC
thr val thr val ser ala ala ser thr lys gly pro ser val phe pro leu ala pro ser
141     151
TCC AAG AGC ACC TCT GGG GGC ACA GCG GCC CTG GGC TCC CTG GTC AAG GAC TAC TTC CCC
ser lys ser thr ser gly gly thr ala ala leu gly cys leu val lys asp tyr phe pro
161     171
GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG
glu pro val thr val ser trp asn ser gly ala leu thr ser gly val his thr phe pro
181     191
GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC
ala val leu gln ser ser gly leu tyr ser leu ser ser val val thr val pro ser ser
201     211
AGC TTG GGC ACC CAG ACC TAC ATC TGC AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG
ser leu gly thr gln thr tyr ile cys asn val asn his lys pro ser asn thr lys val
221     231
GAC AAG AAA GTT GAG CCC AAA TCT TGT GAC AAA act agt
asp lys lys val glu pro lys ser cys asp lys thr ser
```

Fig. 2A

Human Antibody Sequence (TT sequence) (SEQ ID NO: 55)

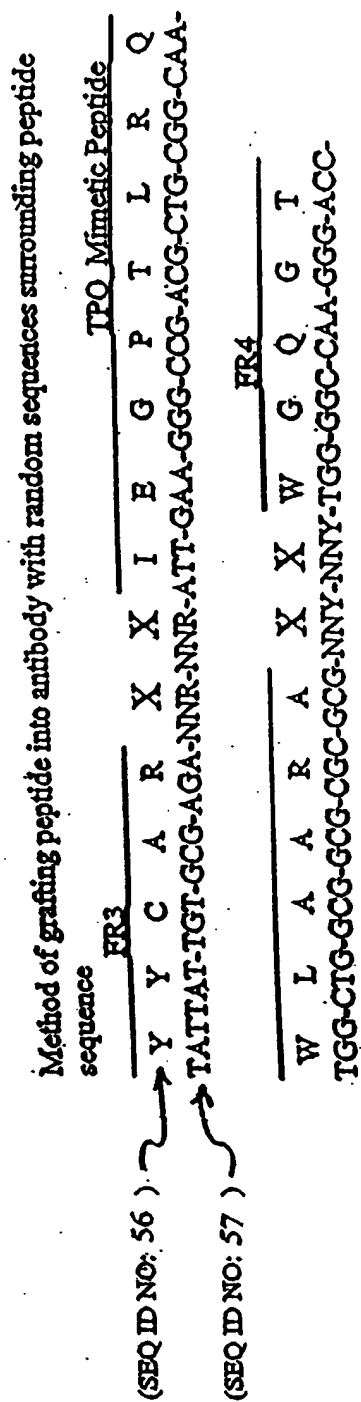
Light Chain: cloning sites Sac I and Xba I are underlined

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1      11
gag ctc acg cag tct cca ggc acc ctg tct ttg tct cca ggg gaa aga gcc acc ctc tcc
glu leu thr gln ser pro gly thr leu ser leu ser pro gly glu arg ala thr leu ser
21      31
tgc agg gcc agt cac agt gtt agc agg gcc tac tta gcc tgg tac cag cag aaa cct ggc
cys arg ala ser his ser val ser arg ala tyr leu ala trp tyr gln gln lys pro gly
41      51
cag gct ccc agg ctc ctc atc tat ggt aca tcc agc agg gcc act ggc atc cca gac agg
gln ala pro arg leu leu ile tyr gly thr ser ser arg ala thr gly ile pro asp arg
61      71
ttc agt ggc agt ggg tct ggg aca gac ttc act ctc acc atc agc aga ctg gag cct gaa
phe ser gly ser gly ser gly thr asp phe thr leu thr ile ser arg leu glu pro glu
81      91
gat ttt gca gtg tac tac tgt cag cag tat ggt ggc tca ccg tgg ttc ggc caa ggg acc
asp phe ala val tyr tyr cys gln gln tyr gly gly ser pro trp phe gly gln gly thr
101     111
AAG GTG GAA CTC AAA CGA ACT GTG GCT GCA CCA TCT GTC TTC ATC TTC CCG CCA TCT GAT
lys val glu leu lys arg thr val ala ala pro ser val phe ile phe pro pro ser asp
121     131
GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA
glu gln leu lys ser gly thr ala ser val val cys leu leu asn asn phe tyr pro arg
141     151
GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC CAG GAG AGT
glu ala lys val gln trp lys val asp asn ala leu gln ser gly asn ser gln glu ser
161     171
GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC ACC CTG ACG CTG AGC
val thr glu gln asp ser lys asp ser thr tyr ser leu ser ser thr leu thr leu ser
181     191
AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC
lys ala asp tyr glu lys his lys val tyr ala cys glu val thr his gln gly leu ser
201     211
TTG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG TGT TAG Ttc tag a
leu pro val thr lys ser phe asn arg gly glu cys AMB

```

Fig. 2B



The TPO mimetic peptide was grafted into the heavy chain CDR3 region of the tetanus toxoid antibody. The peptide was flanked on either side by two random amino acids, shown as "X"s in the figure.

Fig. 3

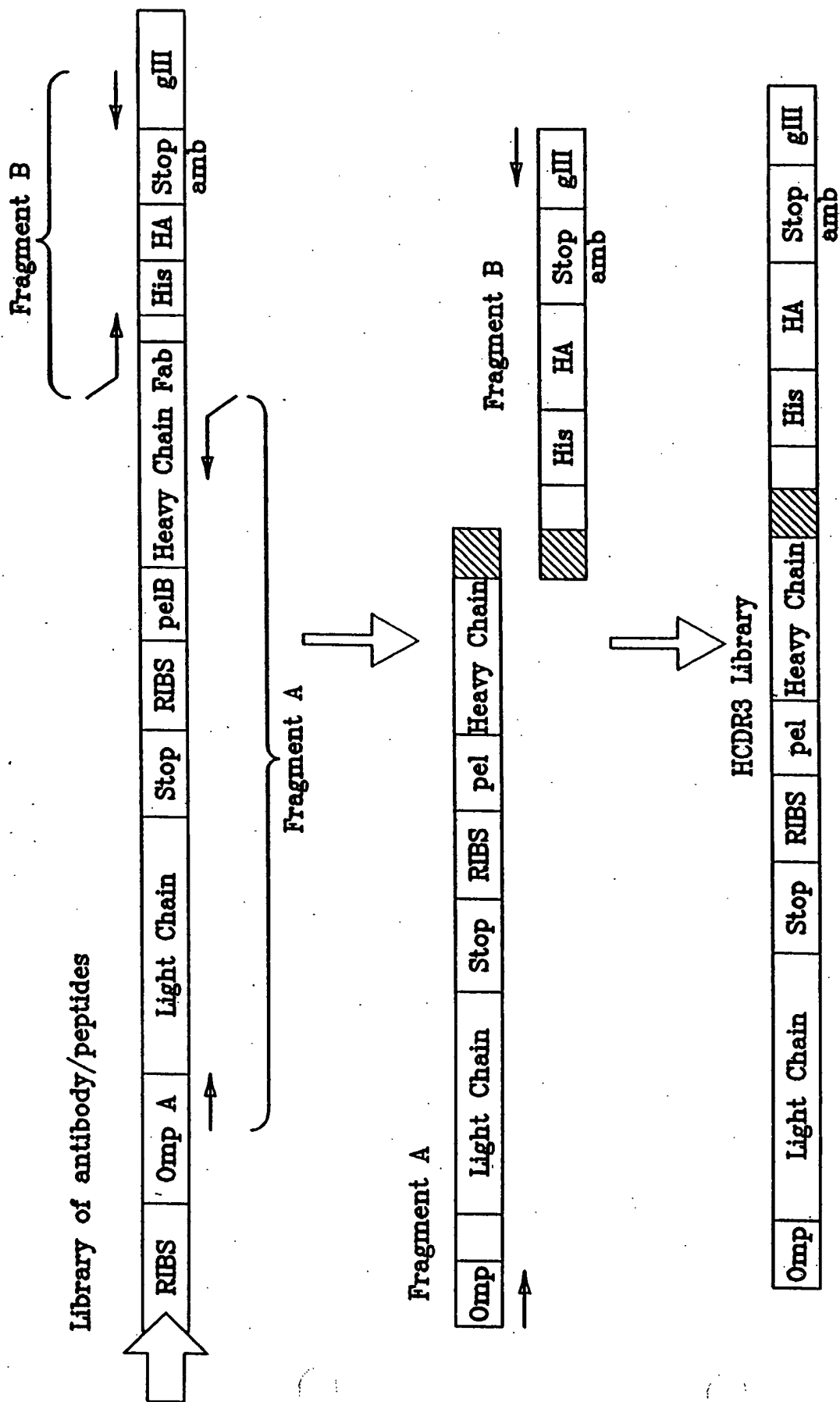


FIG. 4

SEQ ID NO.

AMINO ACID SEQUENCE

CLONE

X1a	Pro-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Gly-Gly CCG-CCC-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCG-GGA-GGC	25 26
X1a-11	Gly-Gly-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Gly-Gly GGG-GGT-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCG-GGA-GGC	27 28
X1a-13	Gly-Gly-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Gly-Gly GGC-GGT-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCG-GGA-GGC	29 30
X1c	Trp-Leu-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val TGG-CTG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCG-CCT-GTC	31 32
X2c	Met-Ile-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Val-Gly ATG-ATA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCG-GTT-GGC	33 34
X3a	Val-Val-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val GTG-GTA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCG-CCT-GTT	35 36
X3b	Gly-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Asp GGG-CCG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCG-CCC-GAT	37 38
X4b	Leu-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val TTG-CCA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCG-CCT-GTT	39 40
X4c	Ser-Leu-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Ile TCA-CTG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCG-CCC-ATC	41 42
X5a	Thr-Met-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val ACA-ATG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCG-CCC-GTT	43 44
X5c	Thr-Thr-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Val ACG-ACA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCG-CCT-GTC	45 46
X7a	Thr-Arg-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Cys-Ser ACA-CGG-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCG-TGC-AGC	47 48
X7b	no peptide deletion mutant	
X7c	Gln-Thr-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Asp CAG-ACA-ATT-GAA-GGG-CCG-ACG-CTG-CGG-CAA-TGG-CTG-GCG-GCG-GCG-CCT-CAC	49 50

Fig. 5

pRL8

(SEQ ID NO: 60)

GGGAAATTGTAAGCGTTAATATTTTGTAAAAATTCCGCGTTAAATTTTGTGTTAA.
ATCAGCTCATTTTTAAACCAATAGGCCGAAATCGGCAAAATCCCTTATAAAATC
AAAAGAATAGACCGAGATAGGGTTGAGTGTGTTCCAGTTTGGAACAAGAGT
CCACTATTAAGAACGTGGACTCCAACGTCAAAGGGCGAAAAACCGTCTATC
AGGGCGATGGCCCACTACGTGAACCATCAACCTAATCAAGTTTTTTGGGGTC
GAGGTGCCGTAAAGCACTAAATCGGAACCCCTAAAGGGAGCCCCCGATTAGAG
GCTTGACGGGGAAAGCCGGCGAACGTGGCGAGAAAGGAAGGGAAAGAAAGC
GAAAGGAGCGGGCGCTAGGGCGCTGGCAAGTGTAGCGGTACGCTGCGCGT
AACCACCAACCCCGCCGCGCTTAATGCGCGCTACAGGGCGCGTCAGGTGGC.
ACTTTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTCTAAATACA
TTCAAATATGTATCCGCTCATGAGACAATAACCCCTGATAAATGCTTCAATAAT
ATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCGCCCTTATTCCC
TTTTTTGCGGCATTTTGCCTTCCTGTTTTTGTCTACCCAGAAACGCTGGTGAAA
GTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTACATCGAACTGG
ATCTCAACAGCGGTAAAGATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCA
ATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTATCCCGTATTGA
CGCCGGGCAAGAGCAACTCGGTGCGCGCATACACTATTCTCAGAATGACTTG
GTTGAGTACTACCAAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAA
GAGAATTATGCAGTGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTT
ACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCCTTTTTTGCACAAC
ATGGGGGATCATGTAACTCGCTTGATCGTTGGGAACCGGAGCTGAATGAAG
CCATACCAAACGACGAGCTGTACACCACGATGCCTGTAGCAATGGCAACAAC
GTTGCGCAAACTATTAACCTGGCGAACTACTTACTCTAGCTTCCCGGCAACAAT
TAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGC
CCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGT
CTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGT
AGTTATCTACACGACGGGGAATCAGGCAACTATGGATGAACGAAATAGACA
GATCGCTGAGATAGGTGCCTCACTGATTAAGCATTGGTAACTGTCAGACCAA
GTTTACTCATATATACTTTAGATTGATTTAAAACCTTCATTTTAAATTTAAAGG
ATCTAGGTGAAGATCCTTTTGATAATCTCATGACCAAAATCCCTTAACGTGA
GTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTT
GAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAAACCACCG
CTACCAGCGGTGGTTTGTGTTGCCGGATCAAGAGCTACCAACTCTTTTTCCGAA
GGTAACTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCCTTCTAGTGTAG
CCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCCTACATACCTCGC
TCGTCTAATCCTGTTACCAAGTGGCTGCTGCCAGTGGCGATAAGTCGTGTCTTA
CCGGGTTGGAATCAAGACGATAGTTACCGGATAAGGCGCAGCGGTCCGGGCTG
AACGGGGGGTTTCGTGCACACAGCCCAGCTTGGAGCGAACGACCTACACCGA

Fig. 6A

ACTGAGATACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGG
 AGAAAGGCGGACAGGTATCCGGTAAGCGGCAGGGTCGGAACAGGAGAGCGC
 ACGAGGGAGCTTCCAGGGGAAACGCCTGGTATCTTTATAGTCCTGTCCGGT
 TTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGG
 AGCCTATGGAAAAACGCCAGCAACCGCGCCTTTTTACGGTTCCTGGCCTTTTG
 CTGGCCTTTTGCTCACATGTTCTTTCTGCGTTATCCCTGATTCTGTGGATAA
 CCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGACGCCGAACGACC
 GAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAA
 CCGCCTCTCCCCGCGCGTTGGCCGATTCATTAATGCAGCTGGCACGACAGGTT
 TCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTAGCTC
 ACTCATTAGGCACCCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGTG
 TGGAATTGTGAGCGGATAACAATTGAATTCAGGAGGAATTTAAAAATGAAAA
 GACAGCTATCGCGATTGCAGTGGCACTGGCTGGTTTCGCTACCGTGGCCAG
 GCGGCCGAGCTCGGCCATGGCTGGTTGGGCAGCGAGTAATAACAATCCAGCG
 GCTGCCGTAGGCAATAGGTATTTTATTATGACTGTCTCCTTGGCGACTAGCTA
 GTTTAGAATTCGTAATCATGGTCATAGCTGTTTCTGTGTGAAATTGTTATCC
 GCTCACAATTCACACAACATACGAGCCGGAAGCATAAAGTGTAAGCCTGG
 GGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGC
 TTTCCAGTCGGGAAACCTGTCTGTTACTAATGATGGTGATGGTGATGGCTAG
 TTTTGTCAAGATTGCGGCTCAACTTTCTTGTCCACCTTGGTGTTGCTGGGCT
 TGTGATTACGTTGCAGATGTAGGTCTGGGTGCCCAAGCTGCTGGAGGGCAC
 GGTACACACGCTGCTGAGGGAGTAGAGTCCTGAGGACTGTAGGACAGCCGGG
 AAGGTGTGCACGCCGCTGGTCAgGGCGCTGAgTTCCACGACACcGTCGCCGG
 TTCgGGGAAGTAGTCTTGACCAGGCAGCCAGGGCCGCTGTGCCCCCAGAG
 GTGCTCTTGGAGGAGGGTGCCAGGGGGAAGACCGATGGGCCCTTGGTGAG
 GCTGCGGAGACGGTGACCGTGGTACCAGCAGAAACCTGGCCAGGCTCCCAG
 GCTCCTCATCTATGGTACATCCAGCAGGGCCACTGGCATCCCAGACAGGTTT
 AGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGC
 CTGAAGATTTTGCAGTGTACTACTGTGAGCAGTATGGTGGCTCACCGTGGTTC
 GGCCAAGGGACCAAGGTGGAACCTCAAACGAACTGTGGCTGCACCATCTGTCT
 TCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACCTGCCTCTGTGTG
 TGCTGTGTAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGG
 ATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAG
 CAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGA
 CTACGAGAAACACAAAGTCTACGCCTGCGAAGTCacccatcaggcgctgagttgccccgtcac
 aaagagcttcaacggaggagtgtaarTCTAGATAATTAATTAGGAGGAATTTAAAAATGAA
 ATACCTATTGCCTACGGCAGCCGCTGGATTGTTATTACTCGCTGCCCCAACCAG
 CCATGGCCGAGGTGCAGCTGCTCGAGATGAGCGATAAAATTATTCACCTGAC
 TGACGACAGTTTTGACACGGATGTACTCAAAGCGGACGGGGCGATCCTCGTC
 GATTTCTGGGCAGAGTGGTGCGGTCCGTGCAAAATGATCGCCCCGATTCTGG
 ATGAAATCGCTGACGAATATCAGGGCAAACCTGACCGTTGCAAACTGAACAT
 CGATCAAAACCCCTGGCACTGCGCCGAAATATGGCATCCGTGGTATCCCGACT
 CTGCTGCTGTTCAAAAACGGTGAAAGTGGCGGCAACCAAAGTGGGTGCACTTG
 TCTAAAGGTCAGTTGAAAGAGTTCTCTGACGCTAACCTGGCGTACCCGTACG
 ACGTTCCGGACTACGGTTCTACTAGTccgaaaccgtclacccaccgggctcttctgcggtggccgc
 atcggccgtctggaggaaaaagtgaanaccctgaaagcicagaactccgagctggcgctccactgccaacatgctgcgcgaac

Fig. 6B

aggtggcacagctgaaacagaaagtatgaacctggcgggtgtgctagtGGCCAGGCCGGCCAGCACCAT
CACCATCACCATGGCGCATACCCGTACGACGTTCCGGACTACGCTTCTTAGG
AGGGTGGTGGCTCTGAGGGTGGCGGTTCTGAGGGTGGCGGCTCTGAGGGAGG
CGGTTCCGGTGGTGGCTCTGGTTCCGGTGATTTTGATTATGAAAAGATGGCAA
ACGCTAATAAGGGGGCTATGACCGAAAATGCCGATGAAAACGCGCTACAGTC
TGACGCTAAAGGCAAACCTTGATTCTGTCGCTACTGATTACGGTGCTGCTATCG
ATGGTTTCATTGGTGACGTTTCCGGCCTTGCTAATGGTAATGGTGCTACTGGT
GATTTTGCTGGCTCTAATTCCTCAATATTTACCTTCCCTCCCTCAATCGGTTGA
ACCTTTAATGAATAATTTCCGTCAATATTTACCTTCCCTCCCTCAATCGGTTGA
ATGTCGCCCTTTTGTCTTTAGCGCTGGTAAACCATATGAATTTTCTATTGATTG
TGACAAAATAAACTTATTCCGTGGTGTCTTTGCGTTTCTTTTATATGTTGCCAC
CTTTATGTATGTATTTTCTACGTTTGCTAACATACTGCGTAATAAGGAGTCCTA
AGCTAGCTAATTAATTTAAGCGGCCGCAGATCT

Fig. 6C

pRL3

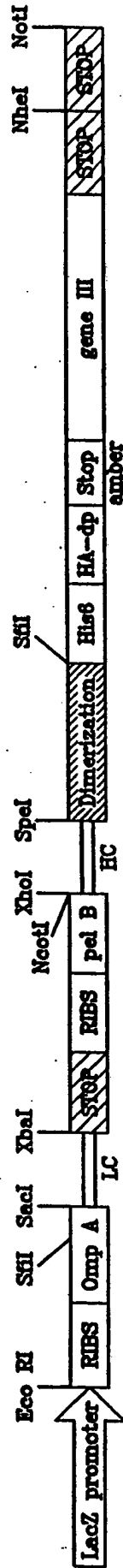


FIG. 7

(SEQ ID NO: 52.) Spe I
 (SEQ ID NO: 53.) → P K P S T P P G S S C G G R I A R L E E K V X T L K A O N S E L A S T A 115
 —hinge— jun dimerization domain—

ACATGCTGCGGACAGGTGGCACAGCTGAAACAGAAAGTTATGAACCATGGCGGTTGTGCTAGTGGCCAGCCGCCAGCACCATCACCATCGGCGCATACCCGTACCGA 230
 N M L R E Q V A Q L K Q K V M N H G G C A S G O A G O H H H H H G A Y P Y D
 — jun dimerization domain — His 6 Tag —

CGTTCCGGACTAGCGTTCTTAGCAGGGTGGTGGCTCTGAG 270
 V P D Y A S
 — HA tag and Amber stop —

Fig. 8

TPO Positive Clones nnk nnk nnk nnk
 1 2 IEGPTLRQWLAARA 3 4

	Sample	nnk	nnk	nnk	nnk	Amino Acids		Amino Acids	
		1	2	3	4	1	2	3	4
HC CDR3	X1c	tgg	ctg	cct	gtc	Trp	Leu	Pro	Val
	X3a	gtg	gta	cct	gtt	Val	Val	Pro	Val
	X3b	ggg	ccg	ccc	gat	Gly	Pro	Pro	Asp
	X4b	ttg	cca	cct	gtt	Leu	Pro	Pro	Val
	X4c	tca	ctg	ccc	atc	Ser	Leu	Pro	Ile
	X5a	aca	atg	ccc	gtt	Thr	Met	Pro	Val
	X5c	acg	aca	cct	gtc	Trp	Leu	Pro	Val
HC CDR2	X7c	cag	aca	cct	cac	Gln	Thr	Pro	Asp
	24	ctt	tat	tct	aat	Leu	Tyr	Ser	Asn
	39	aot	tac	ttg	cat	Thr	Tyr	Leu	His
	3	agg	atg	ctc	gag	Arg	Met	Leu	Glu
	7	aag	gaa	tct	aag	Lys	Glu	Ser	Lys
	8	gcg	cat	gtg	cag	Ala	His	Val	Gln
	10	cag	gag	att	agt	Gln	Glu	Ile	Ser
	11	cgg	aat	aat	ccg	Arg	Asn	Asn	Pro
	19	cag	cta	aat	tct	Gln	Leu	Asn	Ser
	25	agt	att	ttt	gtc	Ser	Ile	Phe	Val
	28	ggg	ccc	act	agt	Gly	Pro	Thr	Ser
	10	aag	ggt	gtt	agt	Lys	Gly	Val	Ser
LC CDR1	11	cat	ggg	gtg	gct	His	Gly	Val	Ala
	12a	cgt	acg	atg	gct	Arg	Thr	Met	Ala
	12b	cgt	ggt	gtt	aat	Arg	Gly	Val	Asn
	14	cgt	tcg	ctt	gcg	Arg	Ser	Leu	Ala
	16	cgg	ggt	gtt	gcg	Arg	Gly	Val	Ala
	18	agg	acg	gtg	tct	Arg	Thr	Val	Ser
	47	aag	ggg	gtg	gcg	Lys	Gly	Val	Ala
	1	aat	ccg	agg	ggt	Asn	Pro	Arg	Gly
LC CDR2	2	tcg	cct	cgg	agt	Ser	Pro	Arg	Ser
	3	tcg	cct	cgt	acg	Ser	Pro	Arg	Thr
	4	tcg	cct	tgg	cgt	Ser	Pro	Trp	Arg
	5	act	ccg	aat	tgg	Thr	Pro	Asn	Trp
	6	aat	cct	gcg	agg	Asn	Pro	Ala	Arg
	7	aat	ccg	tcg	ggg	Asn	Pro	Ser	Gly
	9	aat	cct	tat	tag	Asn	Pro	Tyr	Stop
	10	aat	ccg	cgg	tct	Asn	Pro	Arg	Ser
	11	aat	ccg	gat	gtg	Asn	Pro	Asp	Val
	12	tcg	ccg	tcg	cgg	Ser	Pro	Ser	Arg
	13	aat	cct	ctg	ttt	Asn	Pro	Leu	Phe
	14	aat	ctt	ggg	tat	Asn	Pro	Gly	Tyr
	15	aat	cct	att	agt	Asn	Pro	Ile	Ser
	16	aat	cct	cag	cgg	Asn	Pro	Gln	Arg
	18	aat	ccg	cgg	acg	Asn	Pro	Arg	Thr
	19	aat	ccg	cgt	ggg	Asn	Pro	Arg	Gly
	20	cat	ttg	aga	ctg	His	Leu	Arg	Leu
	21	aag	tag	att	tat	Lys	Stop	Ile	Tyr
	23	aat	cct	ggt	aag	Asn	Pro	Gly	Lys
	24	aat	cct	cgt	ggg	Asn	Pro	Arg	Gly
	26	aat	cct	aat	gtg	Asn	Pro	Asn	Val
	27	tct	ccg	cgg	gtt	Ser	Pro	Arg	Val
	29	acg	cct	cgg	ggt	Thr	Pro	Arg	Gly
	30	ct	tag	tgg	tgg	Pro	Stop	Trp	Trp

FIG. 9

Fig. 10

Activity of Fab clones containing 2 TPO mimetic peptides

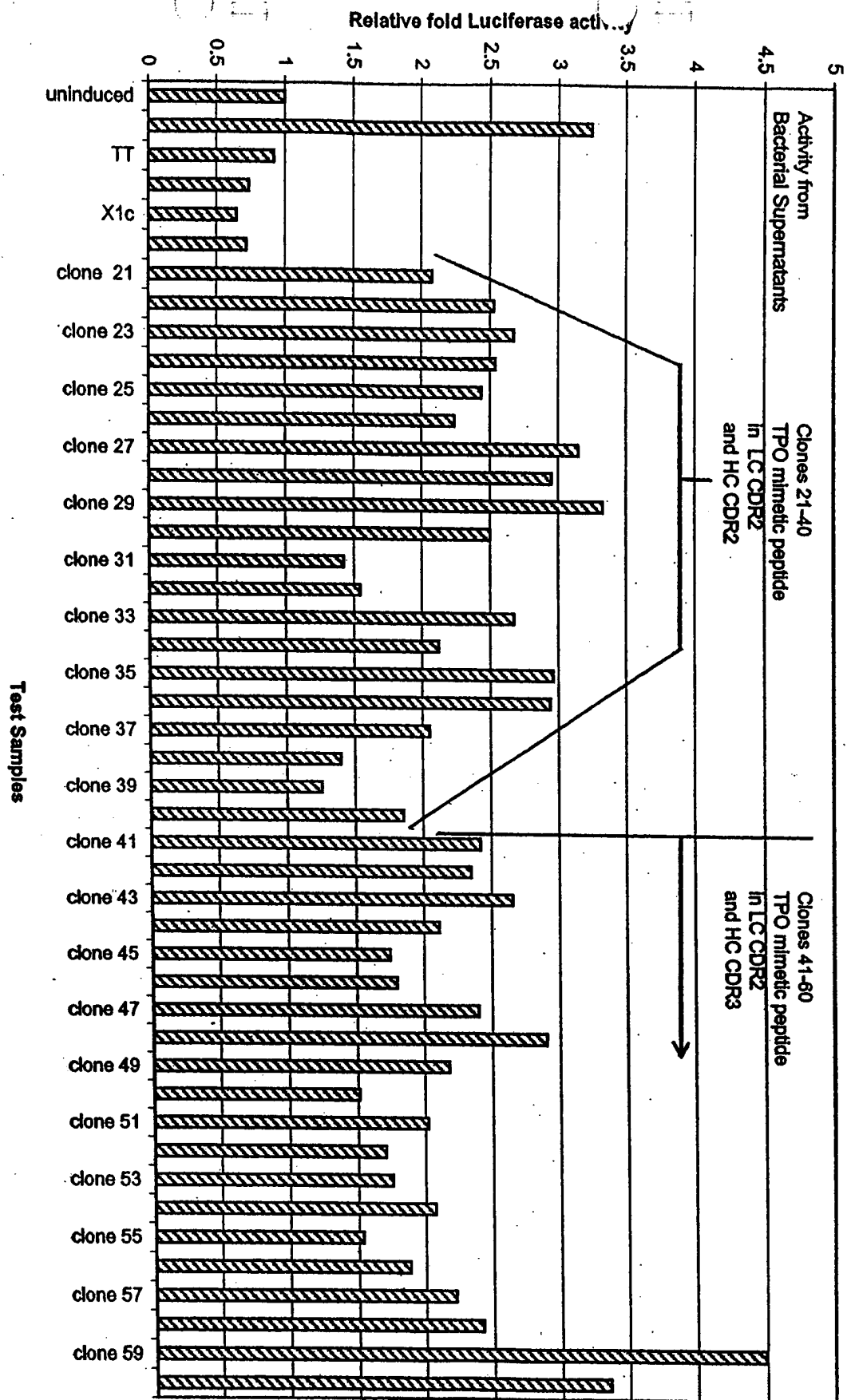
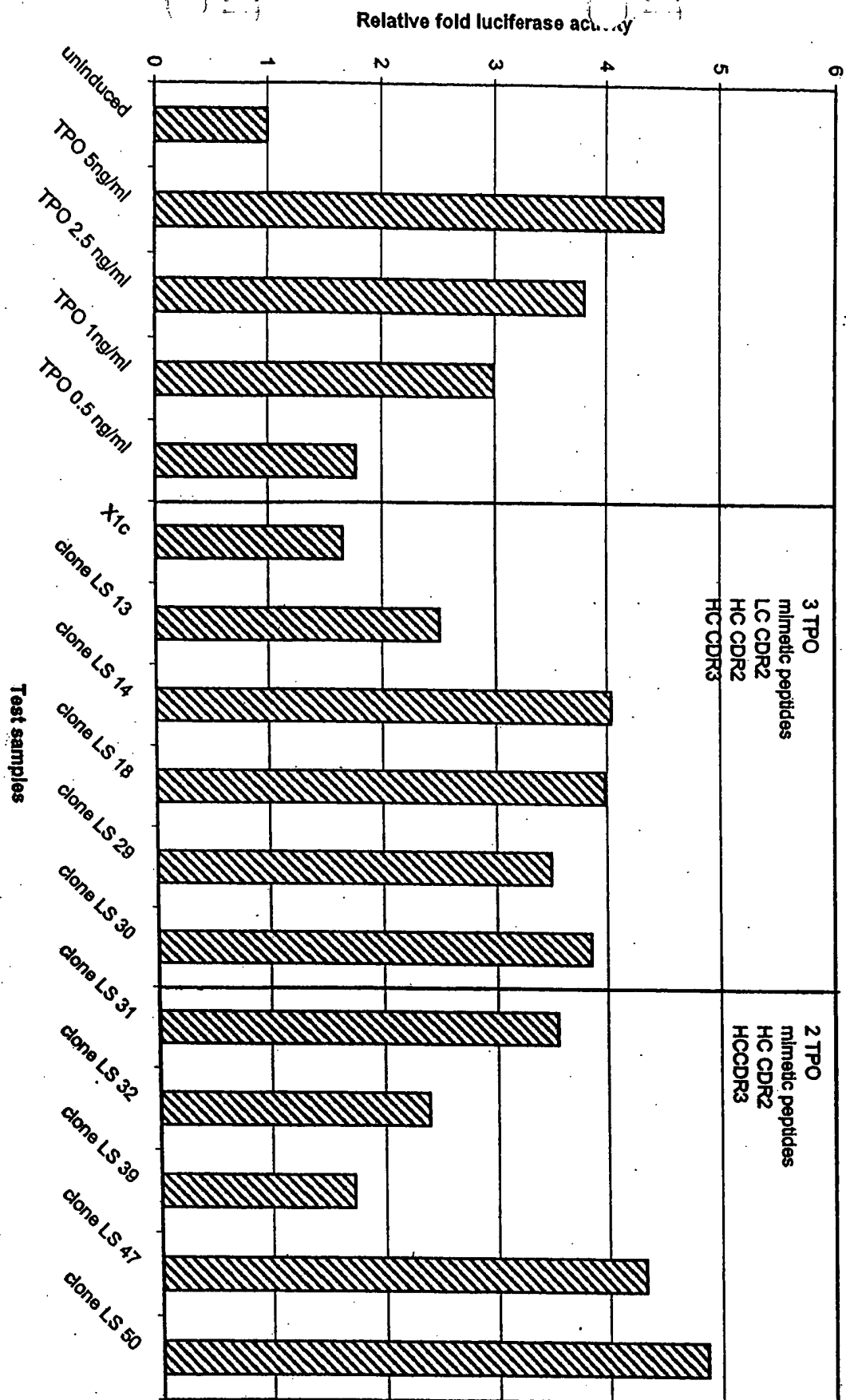
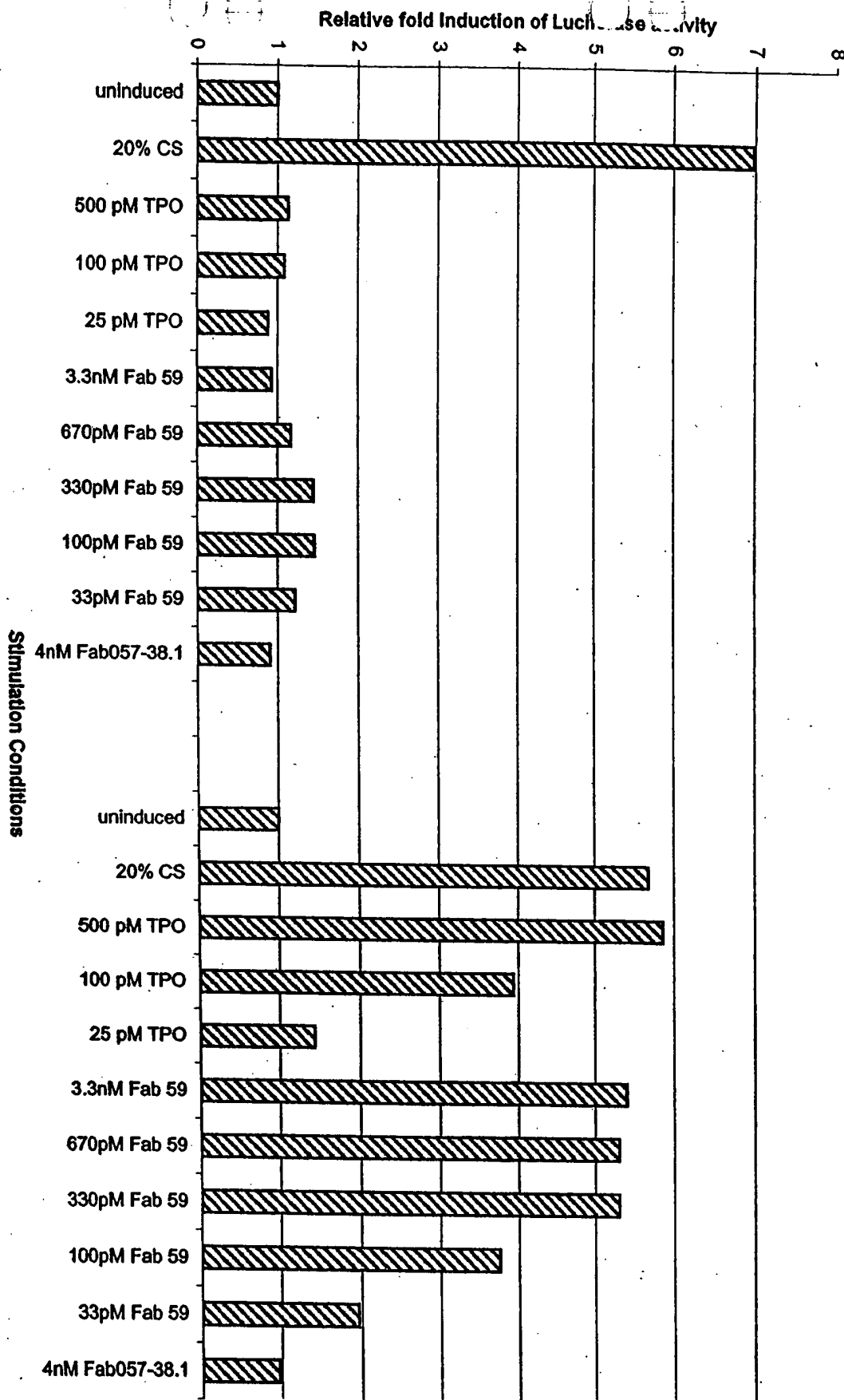


Fig. 11

Activity of Fab clones containing 2 or 3 TPO mimetic peptides





TPO mimetic Activity of semi-purified Fab clone 59

Fig. 12

(SEQ ID NO: 67)

5G1.1 - TPO Heavy Chain (Bold denotes TPO mimetic) Amino acid sequence:

MKW**S**W**V**IL**F**LL**S**VTAG**V**HSQ**V**QL**V**QSGAE**V**KK**P**GA**S**V**K**V**S**CK**A**SG**I**FS**N**Y**W**IQ**W**
VRQ**A**PGQ**G**LE**W**M**G**EIL**P**GS**G**ST**E**Y**T**EN**F**K**D**RV**T**M**R**DT**S**T**S**V**M**EL**S**SL**R**SED
T**A**V**Y**Y**C**AR**L**PI**E**GP**T**LR**Q**W**L**AAR**A**P**V**W**G**Q**T**LV**T**V**S**AS**T**K**G**PS**V**F**L**AP**C**SR
ST**S**EST**A**AL**G**CL**V**K**D**Y**F**PE**P**VT**V**SW**N**SG**A**LT**S**GV**H**TF**A**VL**Q**SS**G**LY**S**LS**S**V**V**TV
P**S**S**N**FG**T**Q**T**Y**T**C**N**VD**H**K**P**SN**T**K**V**DK**T**VER**K**CC**V**EC**P**PC**A**PP**V**AG**P**SV**F**LP**P**K**P**
K**D**TL**M**IS**R**T**P**EV**T**CV**V**VD**V**SQ**E**DP**E**VQ**F**NW**Y**VD**G**VE**V**H**N**AK**T**K**P**REE**Q**FN**S**TY
RV**V**SV**L**TV**L**HQ**D**WL**N**G**K**EY**K**CK**V**SN**K**GL**P**SS**I**E**K**T**I**SK**A**K**G**Q**P**REP**Q**V**T**LP**P**SS**Q**
E**E**MT**K**NQ**V**SL**T**CL**V**K**G**F**Y**PS**D**IA**V**EW**E**SN**G**Q**P**EN**N**Y**K**TP**P**VL**D**SD**G**S**F**FL**Y**S**R**L
TV**D**K**S**R**W**Q**E**GN**V**FS**C**SV**M**HE**A**L**H**N**H**Y**T**Q**K**SL**S**LS**L**SL**G**K.

(SEQ ID NO: 68)

5G1.1 - TPO Heavy Chain (Bold denotes TPO mimetic) Nucleic acid sequence:

AT**G**A**A**G**T**G**G**A**G**C**T**G**G**G**T**A**T**T**C**T**C**T**C**T**C**T**G**T**C**A**G**T**A**A**C**T**G**C**C**G**G**C**T**C**C**A
T**C**C**C**A**A**G**T**C**C**A**A**C**T**G**G**T**G**C**A**A**T**C**C**G**G**C**G**C**C**G**A**G**G**T**C**A**A**G**A**G**C**C**A**G**G**G**G**C**C**
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T**C**A**A**T**G**G**G**T**G**C**G**T**C**A**G**G**C**C**C**C**G**G**C**A**G**G**G**C**T**G**G**A**T**G**G**A**T**G**G**G**T**G**A**G**A**T**C**
T**T**A**C**C**G**G**G**C**T**C**T**G**T**A**G**C**A**C**C**G**A**A**T**A**T**A**C**C**G**A**A**A**T**T**T**T**A**A**A**G**A**C**C**G**T**G**T**A
C**T**A**T**G**A**C**G**C**G**T**G**A**C**A**C**T**T**C**G**A**C**T**A**G**T**A**C**A**G**T**A**T**A**C**A**T**G**G**A**G**C**T**C**C**A**G**C**T**G**
C**G**A**T**C**G**G**A**G**G**A**C**A**C**G**C**C**G**T**C**T**A**T**T**A**T**T**G**C**G**C**G**C**G**T**T**G**C**C**A**A**T**T**G**A**A**G**G**G
C**C**G**A**C**G**C**T**G**C**G**G**C**A**A**T**G**G**C**T**G**G**C**G**C**G**C**G**C**G**C**G**C**T**G**T**T**G**G**G**G**T**C**A**A**G**
G**A**A**C**C**T**G**G**T**C**A**C**T**G**T**C**T**C**G**A**G**C**G**C**T**C**C**A**C**C**A**A**G**G**G**C**C**A**T**C**C**G**T**C**T**C**C**C**
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A**C**T**C**C**T**C**A**G**C**A**G**C**G**T**G**G**T**G**A**C**C**G**T**G**C**C**T**C**C**A**G**C**A**A**C**T**T**C**G**G**C**A**C**C**C**A**G**A**C**
C**T**A**C**A**C**T**G**C**A**A**C**G**T**A**G**A**T**C**A**C**A**G**C**C**C**A**G**C**A**A**C**A**C**A**A**G**G**T**G**G**A**C**A**A**G**A**C**
A**G**T**T**G**A**G**C**G**C**A**A**A**T**G**T**T**G**T**G**T**C**G**A**G**T**G**C**C**C**A**C**C**G**T**G**C**C**C**A**G**C**A**C**A**C**T**G**T**G**
G**C**A**G**G**A**C**C**G**T**C**A**G**T**C**T**C**C**T**C**T**T**C**C**C**C**C**A**A**A**C**C**C**A**A**G**G**A**C**A**C**C**C**T**C**A**T**G**A**T**
C**T**C**C**C**G**A**C**C**C**T**G**A**G**G**T**C**A**C**G**T**G**C**G**T**G**G**T**G**G**T**G**G**A**C**G**T**G**A**G**C**C**A**G**G**A**A**G**A**C**
C**C**C**G**A**G**G**T**C**C**A**G**T**T**C**A**A**C**T**G**G**T**A**C**G**T**G**G**A**T**G**G**C**G**T**G**G**A**G**G**T**G**C**A**T**A**T**G**C**C**A
A**G**A**C**A**A**A**G**C**C**G**C**G**G**A**G**G**A**G**C**A**G**T**T**C**A**A**C**A**G**C**A**C**G**T**A**C**C**G**T**G**T**G**G**T**C**A**G**C**G**
T**C**C**T**C**A**C**C**G**T**C**C**T**G**C**A**C**C**A**G**G**A**C**T**G**G**C**T**G**A**A**C**G**G**C**A**A**G**G**A**G**T**A**C**A**A**G**T**G**C**A**A**
G**G**T**C**T**C**C**A**A**C**A**A**A**G**G**C**T**C**C**C**G**T**C**C**T**C**C**A**T**C**G**A**G**A**A**A**C**C**A**T**C**T**C**C**A**A**A**G**C**C**
A**A**A**G**G**G**C**A**G**C**C**C**G**A**G**A**G**C**C**A**C**A**G**G**T**G**T**A**C**A**C**C**T**G**C**C**C**C**C**A**T**C**C**C**A**G**G**A**G
G**A**G**A**T**G**A**C**C**A**A**G**A**A**C**C**A**G**G**T**C**A**G**C**C**T**G**A**C**T**G**C**C**T**G**G**T**C**A**A**A**G**G**C**T**T**C**T**A**C**C
C**C**A**G**C**G**A**C**A**T**C**G**C**C**G**T**G**G**A**G**T**G**G**G**A**G**A**G**C**A**A**T**G**G**G**C**A**G**C**C**G**G**A**G**A**C**A**A**C**T**
A**C**A**A**G**A**C**C**A**C**G**C**T**C**C**C**G**T**G**G**A**C**T**C**C**G**A**C**G**G**C**T**C**T**T**C**T**C**T**C**T**A**C**A**G**C**
A**G**G**C**T**A**A**C**C**G**T**G**G**A**C**A**A**G**A**G**C**A**G**G**T**G**G**C**A**G**G**A**G**G**G**G**A**A**T**G**T**C**T**T**C**T**C**A**T**G**C**T**
C**C**G**T**G**A**T**G**C**A**T**G**A**G**G**C**T**C**T**G**C**A**A**C**C**A**C**T**A**C**A**C**A**G**A**A**G**A**G**C**C**T**C**C**C**T**

Fig. 13A

(SEQ ID NO: 69)

5G1.1 Light Chain Amino Acid Sequence

MDMRVPAQLLGLLLWLRGARCDIQMTQSPSSLSASVGDRVTITCGASENIYGALN
WYQQKPGKAPKLLIYGATNLADGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQ
NVLNTPITFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAK
VQWKVDNALQSGNSQESVTEQDSKDSYLSSTLTLSKADYEKHKVYACEVTH
QGLSSPVTKSFNRGEC.

(SEQ ID NO: 70)

5G1.1 Light Chain Nucleic Acid Sequence

ATGGACATGAGGGTCCCCGCTCAGCTCCTGGGGCTCCTGCTACTCTGGCTCCG
AGGTGCCAGATGTGATATCCAGATGACCCAGTCCCCGTCCTCCCTGTCCGCCT
CTGTGGGCGATAGGGTCACCATCACCTGCGGCGCCAGCGAAAACATCTATGG
CGCGCTGAACTGGTATCAACAGAAACCCGGGAAAGCTCCGAAGCTTCTGATT
TACGGTGCGACGAACCTGGCAGATGGAGTCCCTTCTCGCTTCTCTGGATCCGG
CTCCGGAACGGATTTCACTCTGACCATCAGCAGTCTGCAGCCTGAAGACTTC
GCTACGTATTACTGTCAGAACGTTTTAAATACTCCGTTGACTTTCGGACAGGG
TACCAAGGTGGAAATAAAACGAACTGTGGCTGCACCATCTGTCTTCATCTTCC
CGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTG
AATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCC
TCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACA
GCACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGA
AACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGT
CACAAAGAGCTTCAACAGGGGAGAGTGTTAG

Note: Italics denotes leader sequence

Fig. 13B

Fig. 14

FACS staining on transfected 293 cells

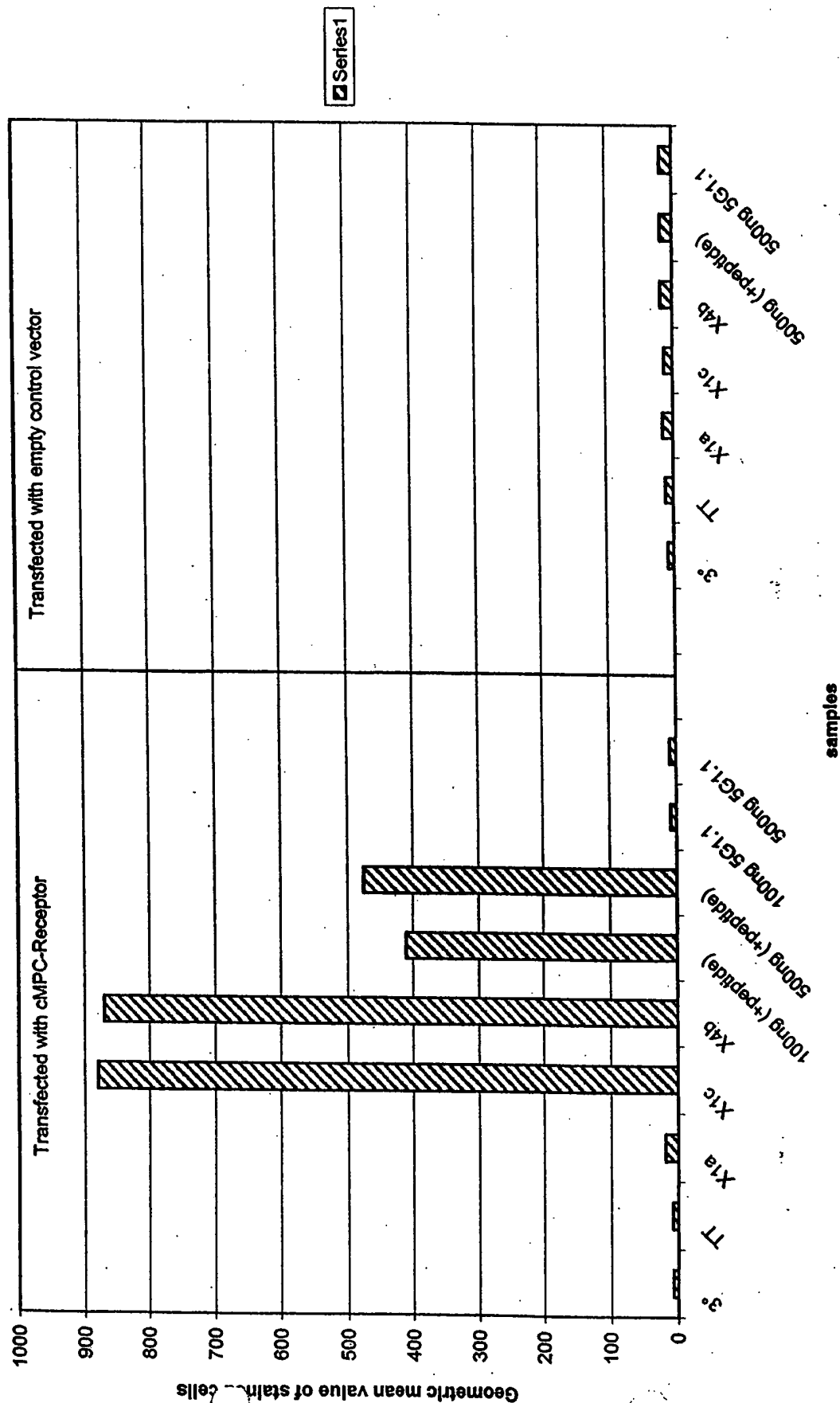
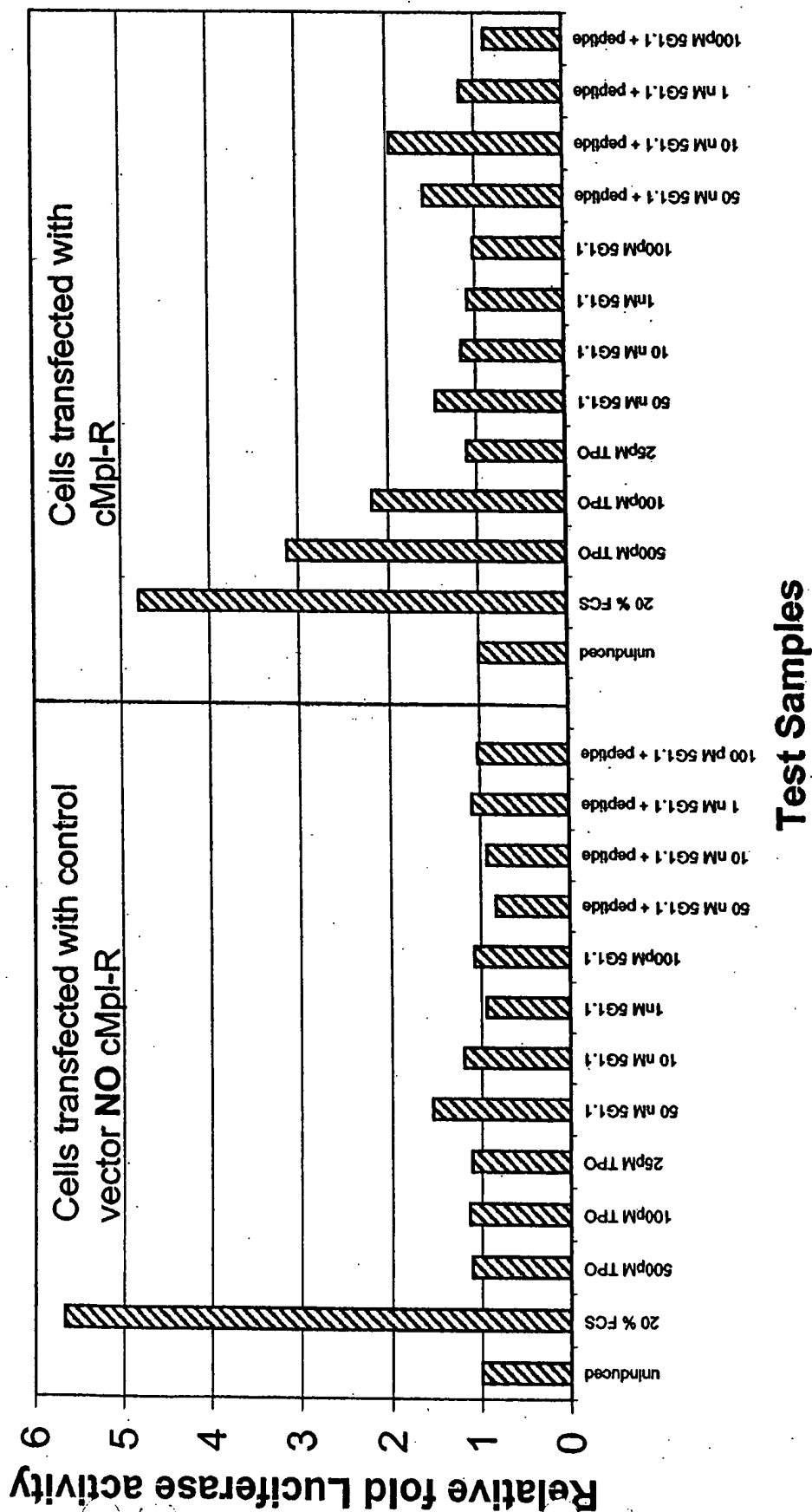


Fig. 15

Activity of 5G1.1 containing the TPO mimetic Peptide



(SEQ ID NO: 116)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100 101 102 103 104 105 106 107 108 109 110 111 112 113 114 115 116 117 118 119 120 121 122 123 124 125 126 127 128 129 130 131 132 133 134 135 136 137 138 139 140 141 142 143 144 145 146 147 148 149 150 151 152 153 154 155 156 157 158 159 160 161 162 163 164 165 166 167 168 169 170 171 172 173 174 175 176 177 178 179 180 181 182 183 184 185 186 187 188 189 190 191 192 193 194 195 196 197 198 199 200 201 202 203 204 205 206 207 208 209 210 211 212 213 214 215 216 217 218 219 220 221 222 223 224 225 226 227 228 229 230 231 232 233 234 235 236 237 238 239 240 241 242 243 244 245 246 247 248 249 250 251 252 253 254 255 256 257 258 259 260 261 262 263 264 265 266 267 268 269 270 271 272 273 274 275 276 277 278 279 280 281 282 283 284 285 286 287 288 289 290 291 292 293 294 295 296 297 298 299 300 301 302 303 304 305 306 307 308 309 310 311 312 313 314 315 316 317 318 319 320 321 322 323 324 325 326 327 328 329 330 331 332 333 334 335 336 337 338 339 340 341 342 343 344 345 346 347 348 349 350 351 352 353 354 355 356 357 358 359 360 361 362 363 364 365 366 367 368 369 370 371 372 373 374 375 376 377 378 379 380 381 382 383 384 385 386 387 388 389 390 391 392 393 394 395 396 397 398 399 400 401 402 403 404 405 406 407 408 409 410 411 412 413 414 415 416 417 418 419 420 421 422 423 424 425 426 427 428 429 430 431 432 433 434 435 436 437 438 439 440 441 442 443 444 445 446 447 448 449 450 451 452 453 454 455 456 457 458 459 460 461 462 463 464 465 466 467 468 469 470 471 472 473 474 475 476 477 478 479 480 481 482 483 484 485 486 487 488 489 490 491 492 493 494 495 496 497 498 499 500 501 502 503 504 505 506 507 508 509 510 511 512 513 514 515 516 517 518 519 520 521 522 523 524 525 526 527 528 529 530 531 532 533 534 535 536 537 538 539 540 541 542 543 544 545 546 547 548 549 550 551 552 553 554 555 556 557 558 559 560 561 562 563 564 565 566 567 568 569 570 571 572 573 574 575 576 577 578 579 580 581 582 583 584 585 586 587 588 589 590 591 592 593 594 595 596 597 598 599 600 601 602 603 604 605 606 607 608 609 610 611 612 613 614 615 616 617 618 619 620 621 622 623 624 625 626 627 628 629 630 631 632 633 634 635 636 637 638 639 640 641 642 643 644 645 646 647 648 649 650 651 652 653 654 655 656 657 658 659 660 661 662 663 664 665 666 667 668 669 670 671 672 673 674 675 676 677 678 679 680 681 682 683 684 685 686 687 688 689 690 691 692 693 694 695 696 697 698 699 700 701 702 703 704 705 706 707 708 709 710 711 712 713 714 715 716 717 718 719 720 721 722 723 724 725 726 727 728 729 730 731 732 733 734 735 736 737 738 739 740 741 742 743 744 745 746 747 748 749 750 751 752 753 754 755 756 757 758 759 760 761 762 763 764 765 766 767 768 769 770 771 772 773 774 775 776 777 778 779 780 781 782 783 784 785 786 787 788 789 790 791 792 793 794 795 796 797 798 799 800 801 802 803 804 805 806 807 808 809 810 811 812 813 814 815 816 817 818 819 820 821 822 823 824 825 826 827 828 829 830 831 832 833 834 835 836 837 838 839 840 841 842 843 844 845 846 847 848 849 850 851 852 853 854 855 856 857 858 859 860 861 862 863 864 865 866 867 868 869 870 871 872 873 874 875 876 877 878 879 880 881 882 883 884 885 886 887 888 889 890 891 892 893 894 895 896 897 898 899 900 901 902 903 904 905 906 907 908 909 910 911 912 913 914 915 916 917 918 919 920 921 922 923 924 925 926 927 928 929 930 931 932 933 934 935 936 937 938 939 940 941 942 943 944 945 946 947 948 949 950 951 952 953 954 955 956 957 958 959 960 961 962 963 964 965 966 967 968 969 970 971 972 973 974 975 976 977 978 979 980 981 982 983 984 985 986 987 988 989 990 991 992 993 994 995 996 997 998 999 1000 1001 1002 1003 1004 1005 1006 1007 1008 1009 1010 1011 1012 1013 1014 1015 1016 1017 1018 1019 1020 1021 1022 1023 1024 1025 1026 1027 1028 1029 1030 1031 1032 1033 1034 1035 1036 1037 1038 1039 1040 1

		Grafted CDR2																																			
		CDR1		29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	52a	52b	52c	52d	52e	52f	52g	52h	52i	52j
ATT	AGT	AGT	AGT	TTG	CTG	CCC	TGG	TAT	CAG	CAG	AAA	CCA	GGG	AAA	GCT	CCT	AAG	CTC	CTG	ATC	TAT	AAC	CCG	ATC	GAA	GGC	CCA	ACC	CTG	COC	CAG	TGG	CTG	GCT			

Grafted CDR2

52k 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85

ACT CGC GCT GGT GGT GGG GTC CCA TCA AGG TTC AGC GGC AGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATC AGC AGC CTG CAG CCT GAA GAT TTT GCA ACT

	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101	102	103	104	105	106	107
TAT	TAC	TGC	CAA	CAG	TAT	AAT	AAT	AGT	TAC	CCT	CCC	ACT	TTC	GGC	CCT	GGG	ACC	AAA	GTG	GAT	ATC	AAA

Fig. 16

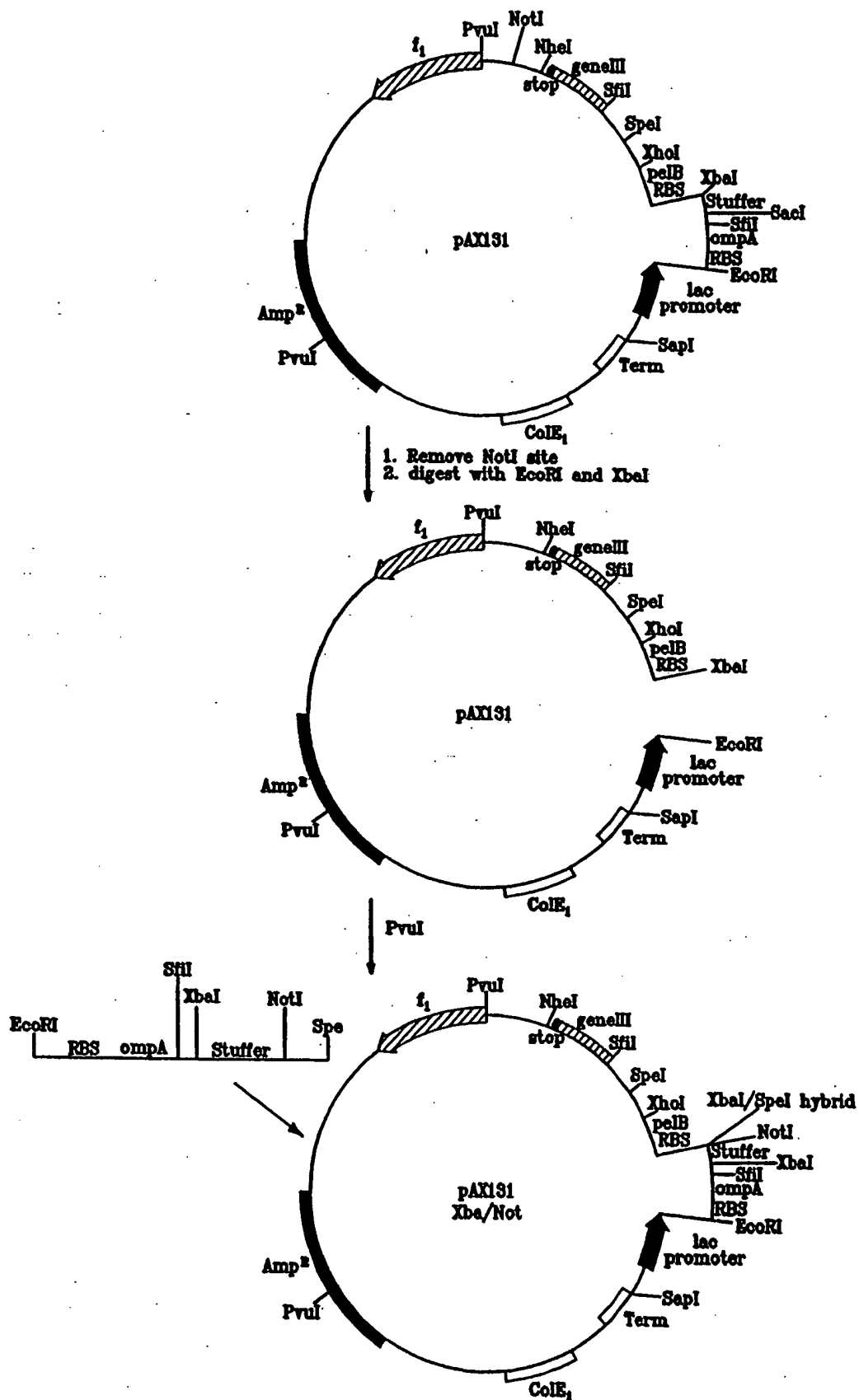


FIG. 17

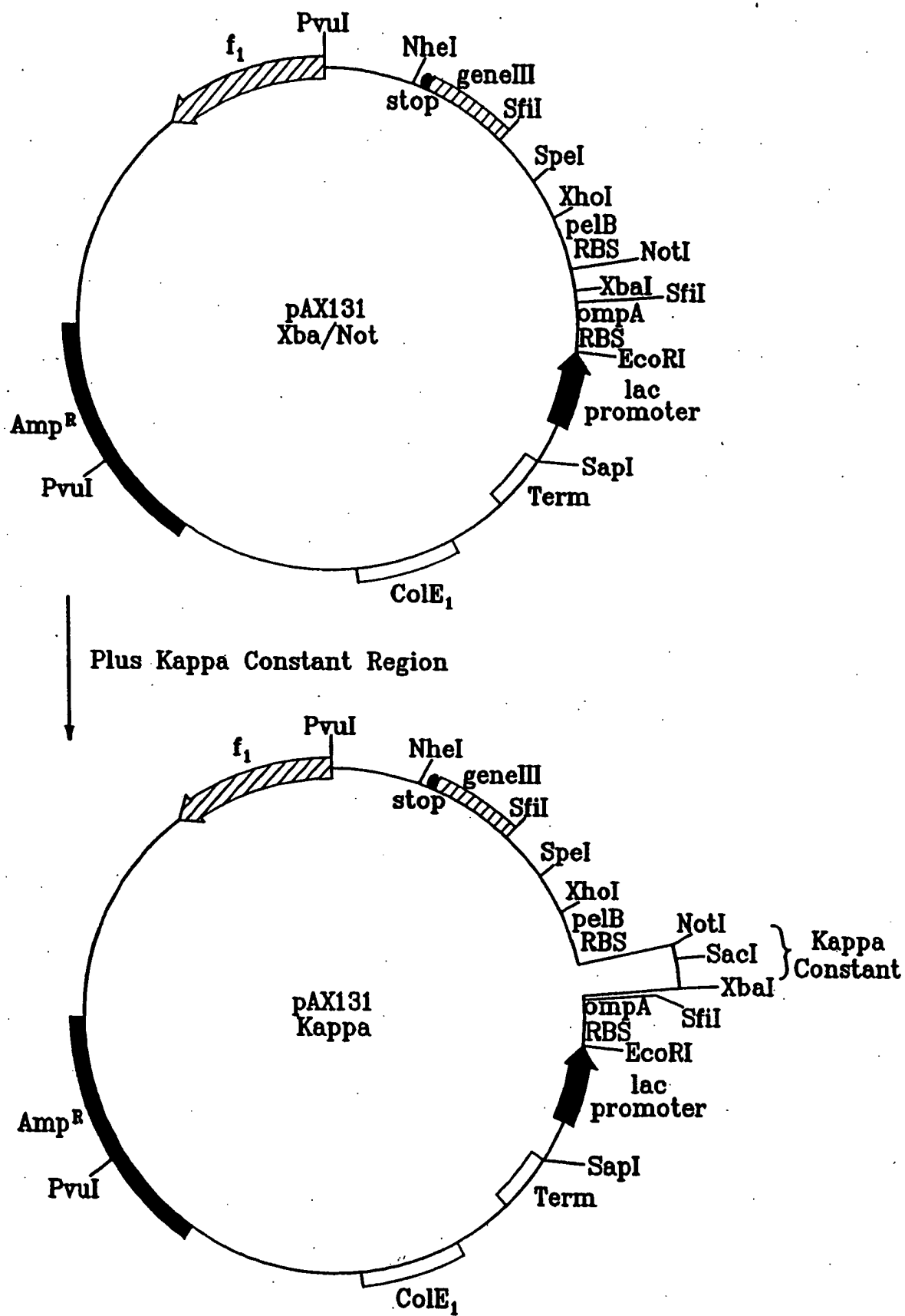


FIG. 18

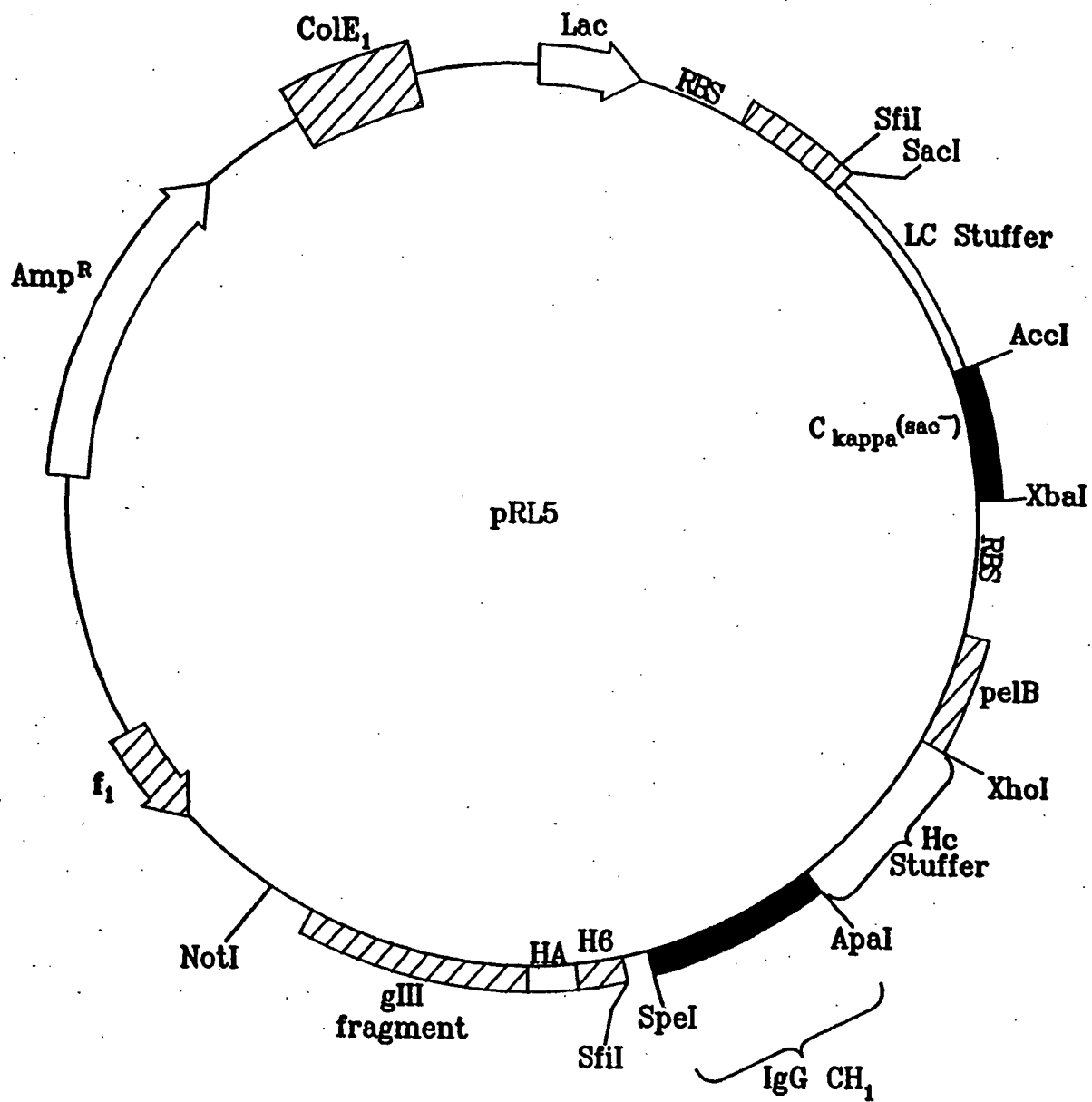


FIG. 19

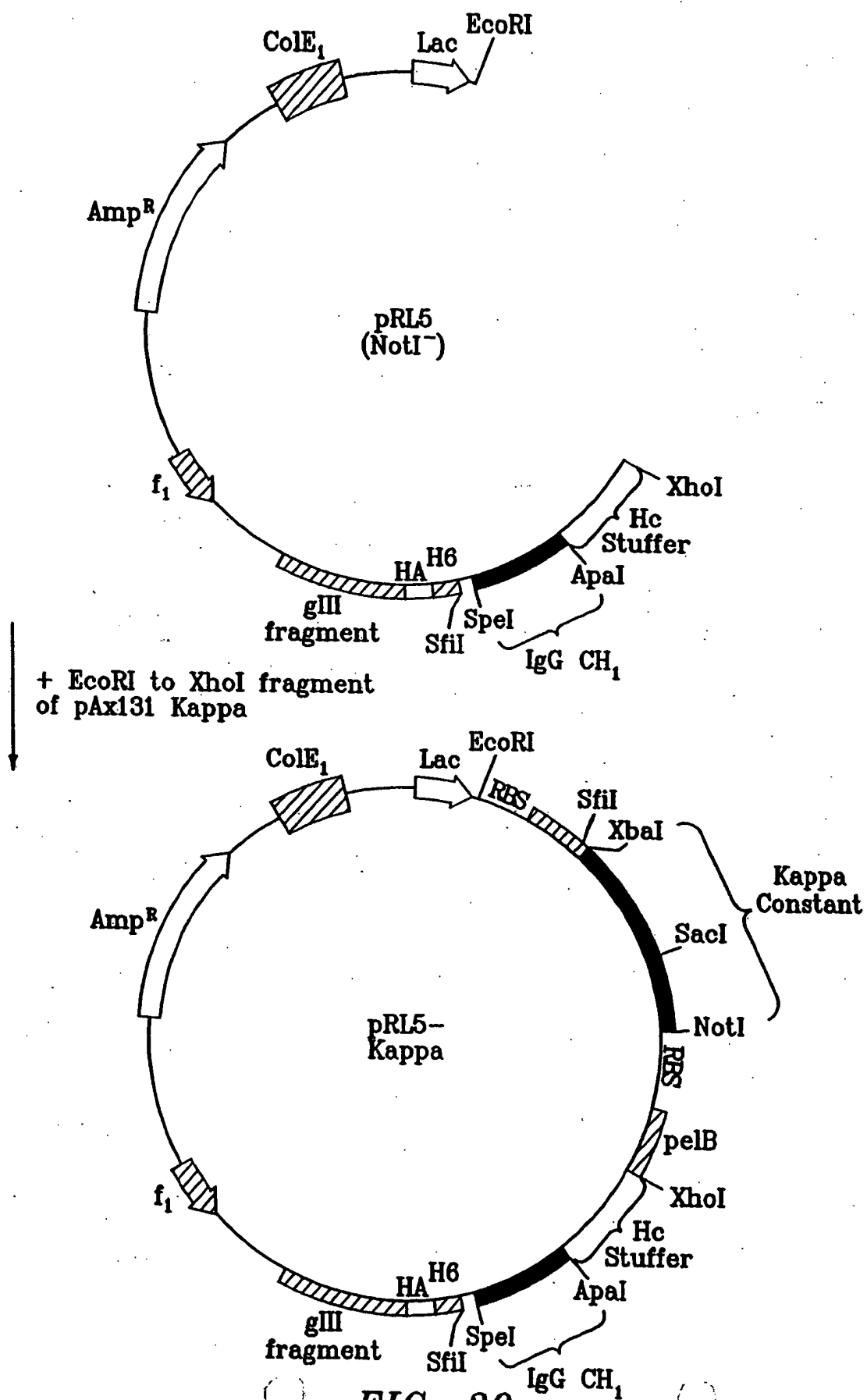


FIG. 20

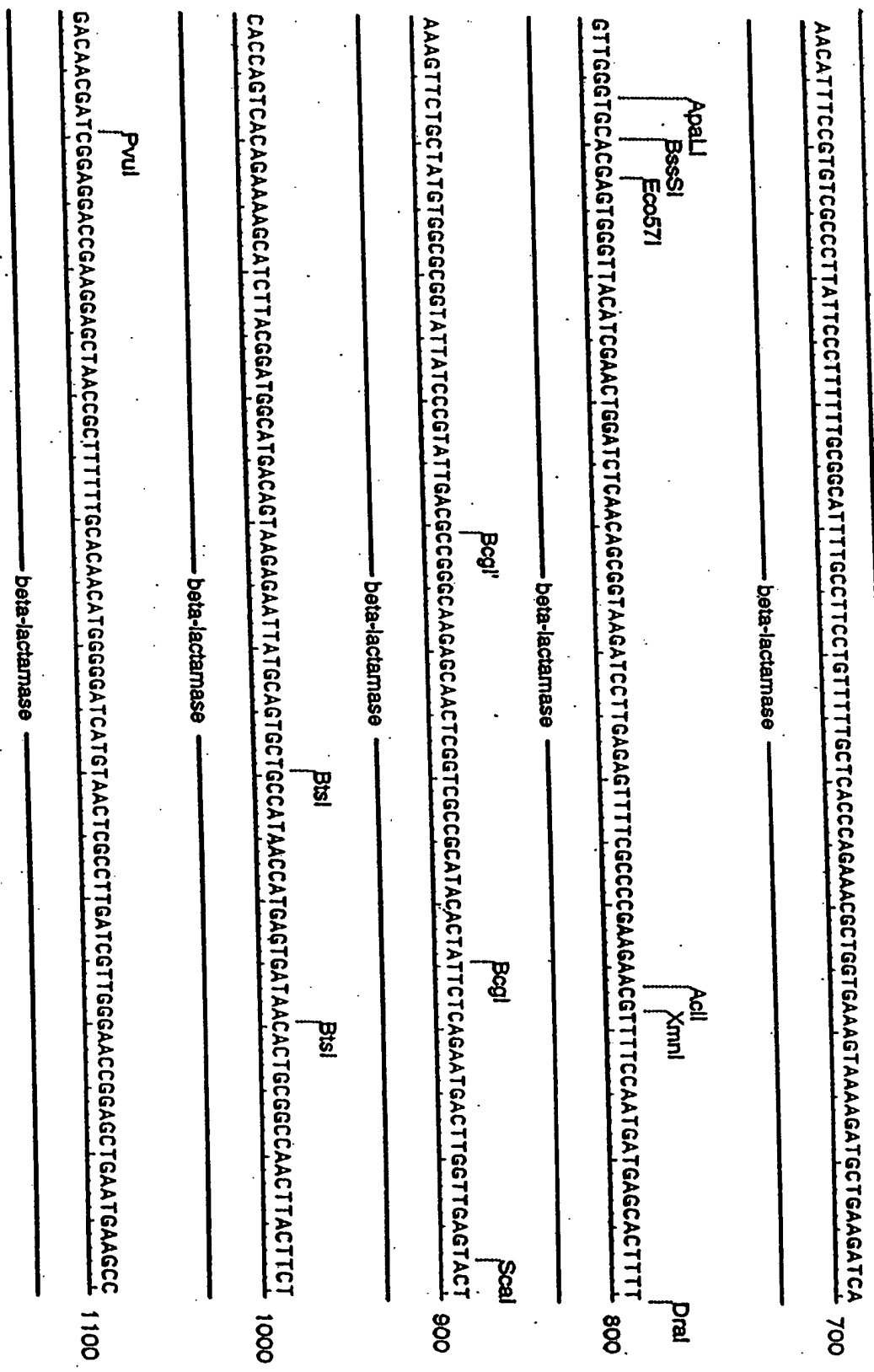


Fig. 21B

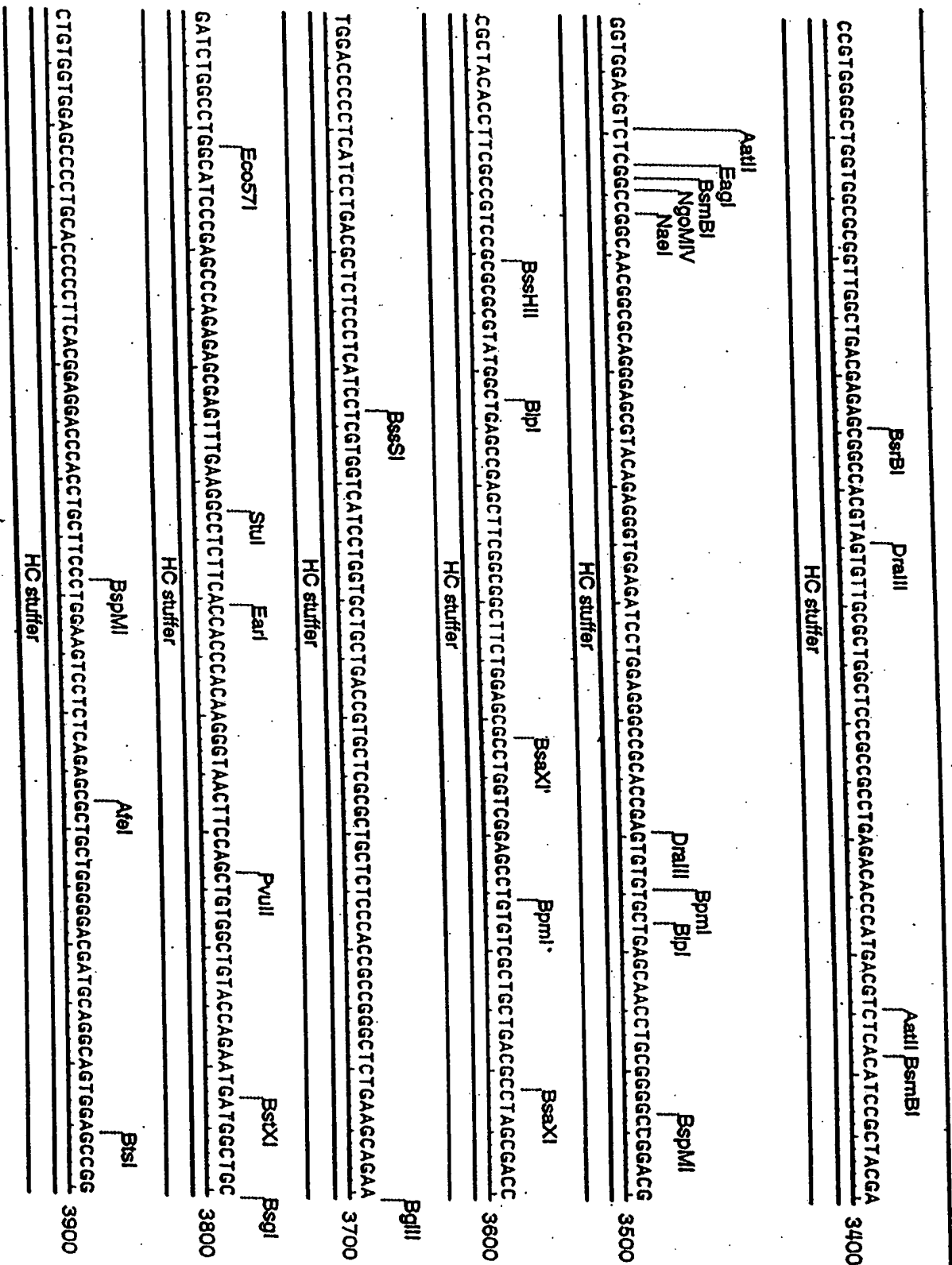


Fig. 21C

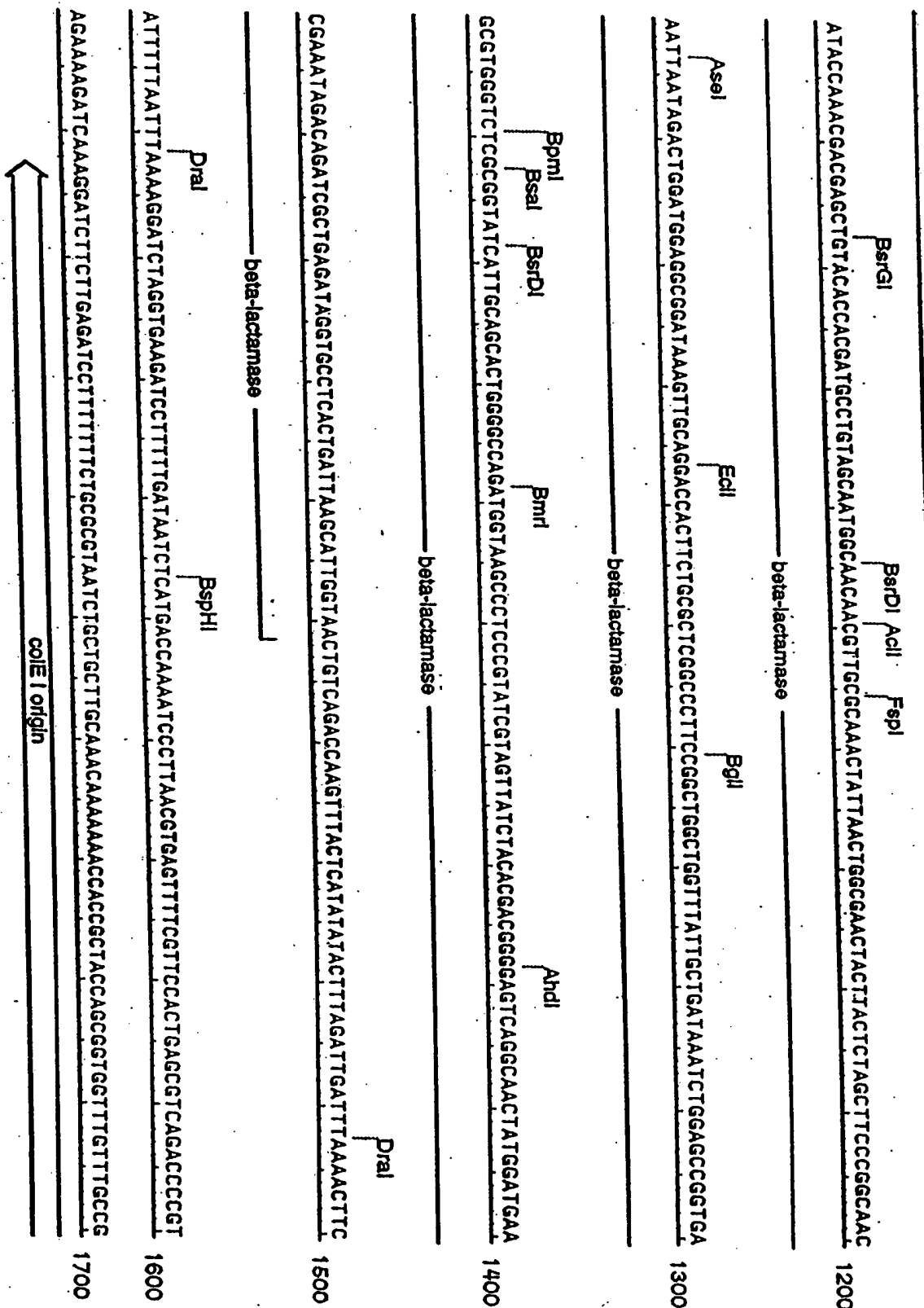
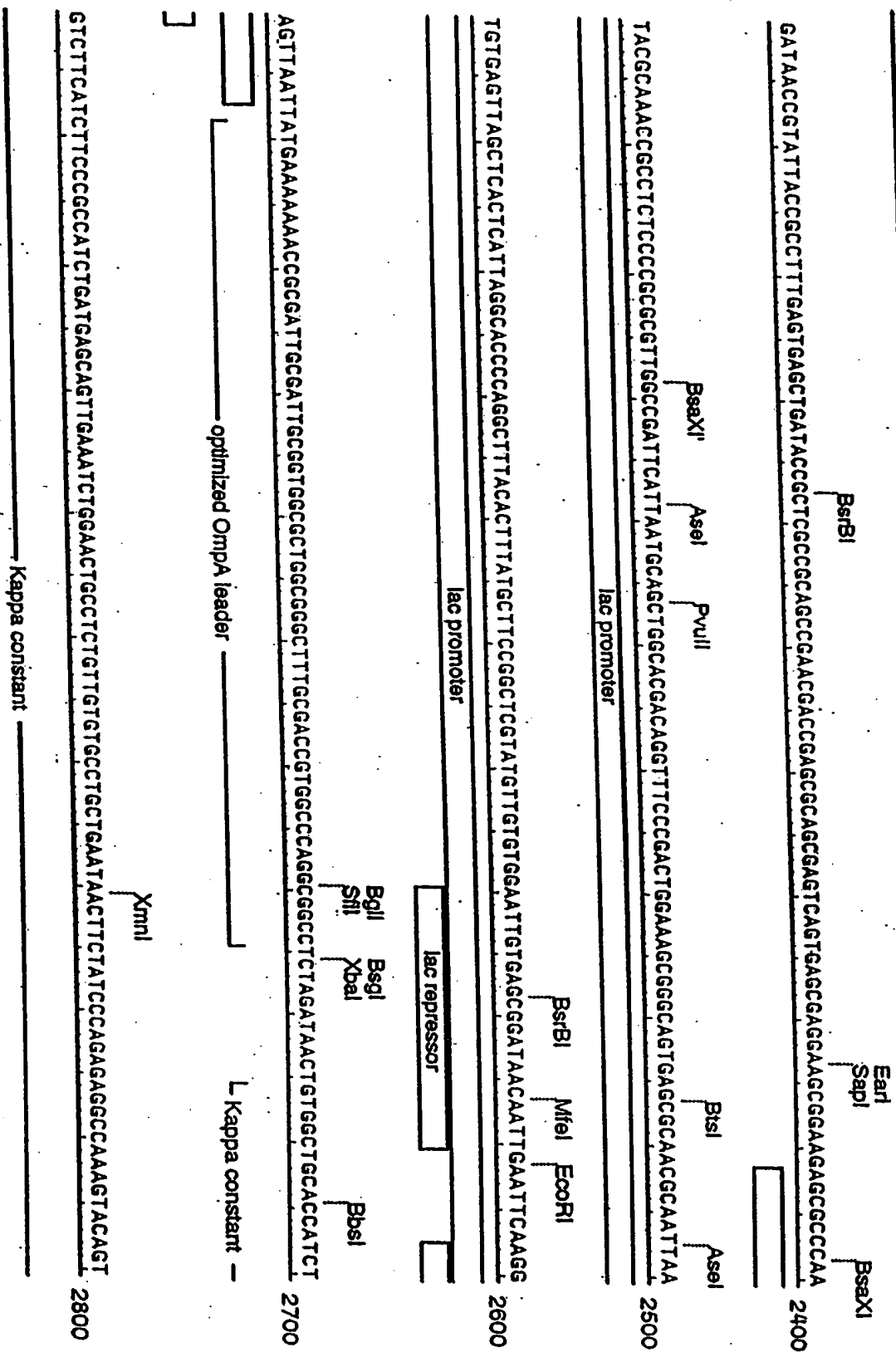
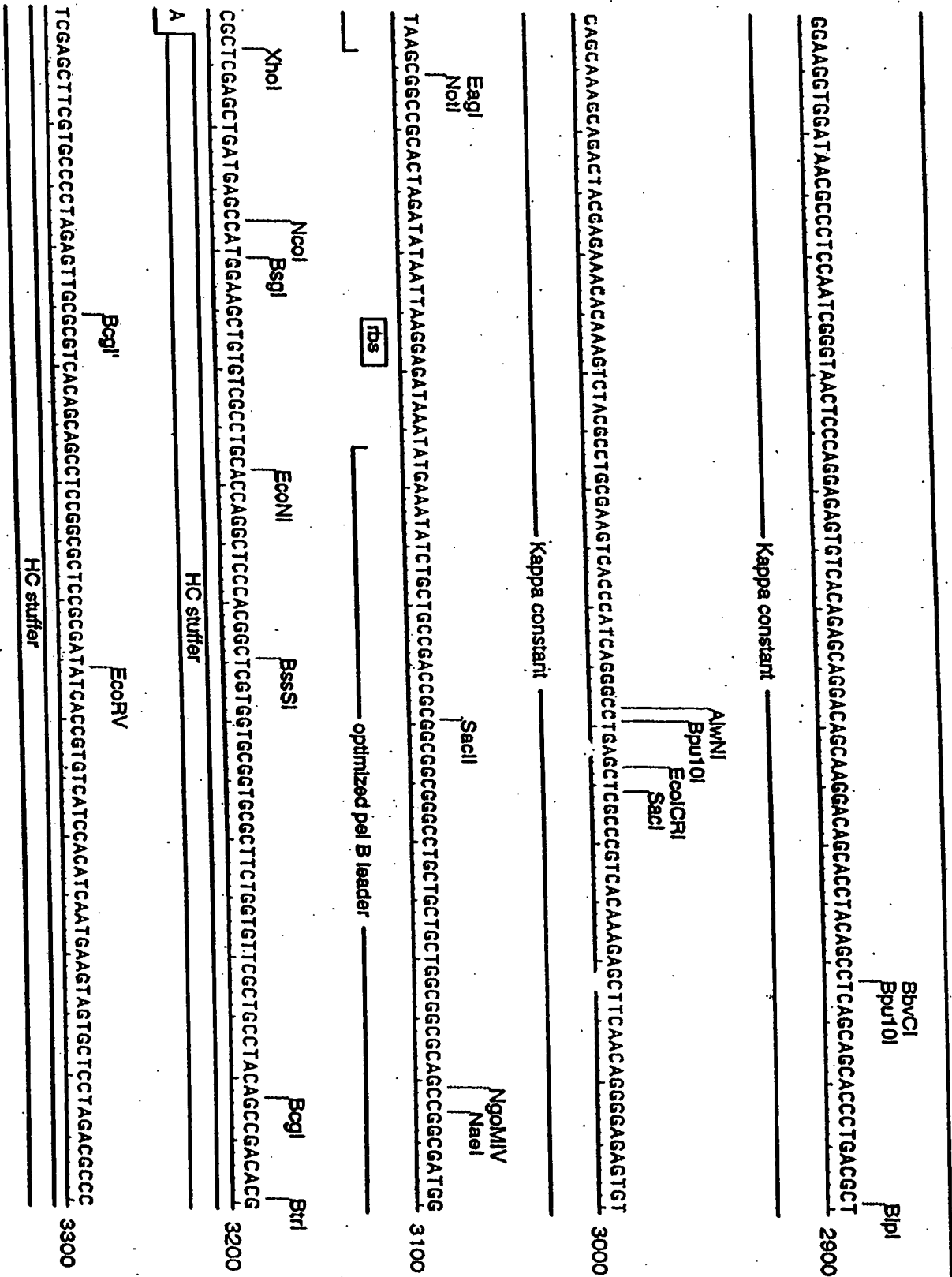


Fig. 21D





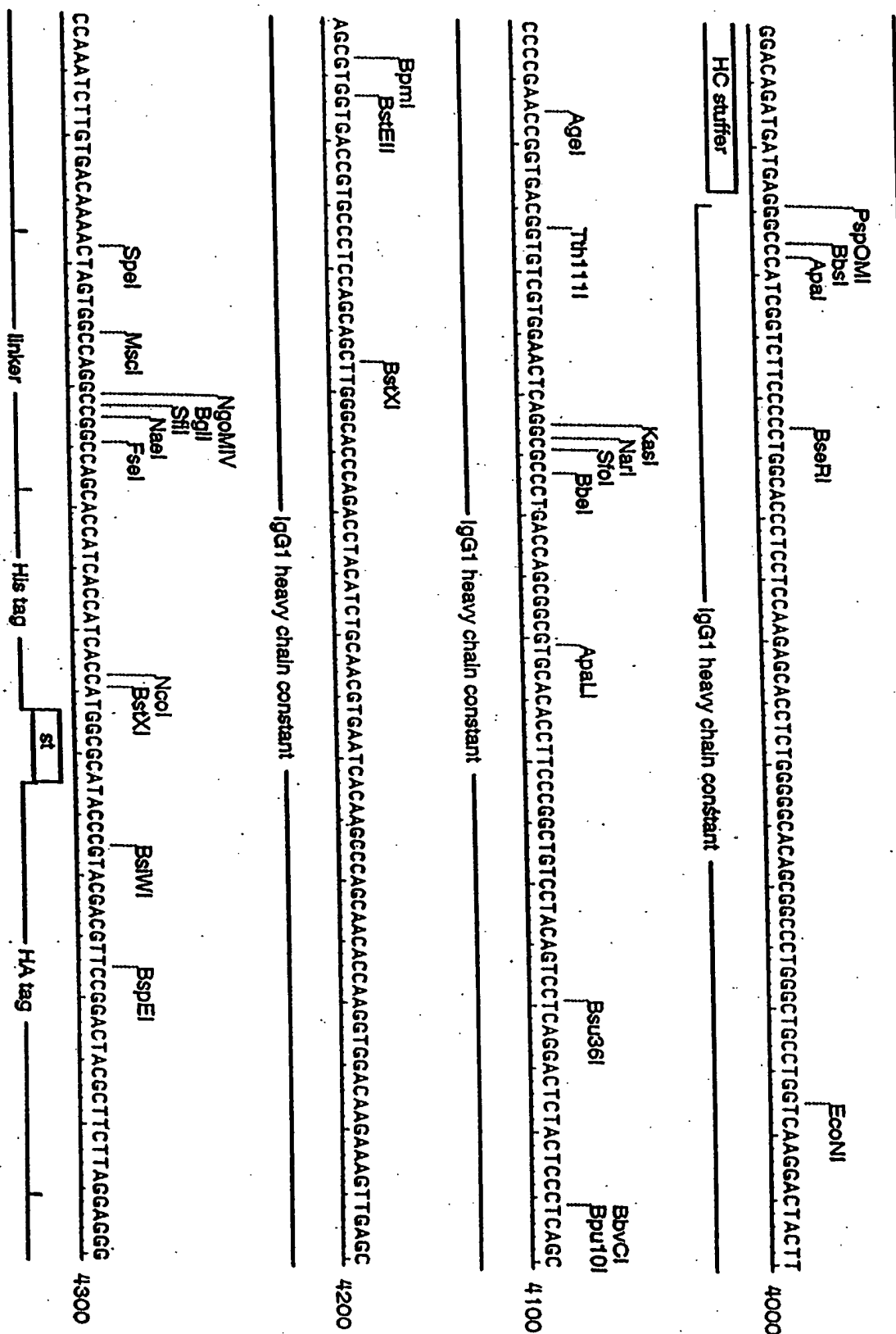


Fig. 21H

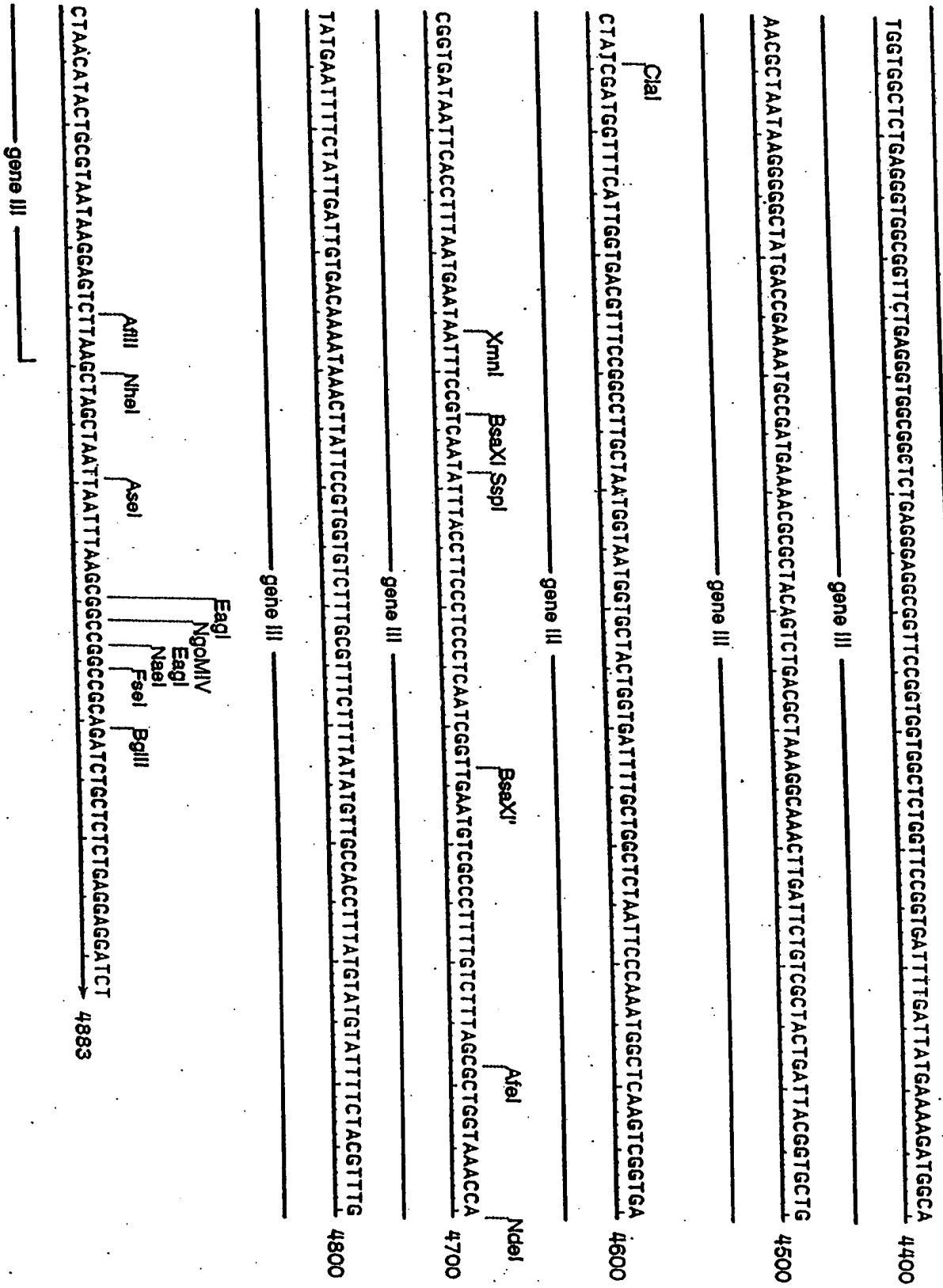


Fig. 21I

Figure 22

VH: L22582 (human germline family member VH1-69)

GCAGGATTTAGGGCTTGGTCTCTCAGCATCCCACACTTGTACAGCTGATGTGGCATCTG
TGTTTTCTTTCTCATCGTAGATCAGGCTTTGAGCTGTGAAATACCCTGCCTCATGCATATGCA
AATAACCTGAGGTCTTCTGAGATAAATATAGATATATTGGTGCCCTGAGAGCATCACATAA
CAACCACATTCTCTCTCTAAAGAAGCCCCCTGGGAGCACAGCTCATCACCATGGACTGGACC
TGGAGGTTCTCTTTGTGGTGGCAGCGCTACAGGTAAGGGGCTTCCTAGTCCTAAGGCTGAG
GAAGGGATCCTGGTTTGTAAAGAGGATTTTATTACCCCTGTGTCTCTCCACAGGTGTC
CAGTCCCAGGTGCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGTCCTCGGTGA
AGGTCTCTGCAAGGCTTCTGGAGGCACCTTCAGCAGCTATGCTATCAGCTGGGTGCGACA
GGCCCCCTGGACAAGGGCTTGAGTGGATGGGAGGGATCATCCCTATCTTTGGTACAGCAAAC
TACGCACAGAAGTTCCAGGGCAGAGTCACGATTACCGCGGACGAATCCACGAGCACAGCCT
ACATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGACAC
AGTGTGAAAAACCCACATCCTGAGAGTGTGAGAAACCTGAGGGAGAAGGCAGCTGTGCCG
GGCTGAGGAGATGACAGGGTTTATTAGGTTTAAGGCTGTTTACAAAATGGGTTATATTTTG
AGAAAAAAGAACAGTAGAAACAAGTACATACTCTCTAATTTTAAGATAATTATTCCATT
CAAGAGTCGTAATAT (SEQ ID NO. 153)

JH---JH6

H3

CDR3

100

110

JH6 YYYYYYGMDVWGQGTIVTVSS (SEQ ID NO 154)

Figure 23

Vk: X12686 (human germline family member VKIII -A27)

CAGCTGCTTTGCATGTCCCTCCCAGCCGCCCTGCAGTCCAGAGCCCATATCAATGCCTGG
 GTCAGAGCTCTGGAGAAGAGCTGCTCAGTTAGGACCCAGAGGGAACCATGGAAACCCAG
 CGCAGCTTCTCTTCCCTCCTGCTACTCTGGCTCCCAGGTGAGGGGAACATGGGATGGTTTTGC
 ATGTCAGTGAAAACCCCTCTCAAGTCCTGTTACCTGGCAACTCTGCTCAGTCAATACAATAAT
 TAAAGCTCAATATAAAGCAATAATTCTGGCTCTTCTGGGAAGACAATGGGTTTGATTAGAT
 TACATGGGTGACTTTTCTGTTTTATTTCCAATCTCAGATACCACGGAGAAATTGTGTTGAC
 GCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCACCCTCTCCTGCAGGGCCA
 GTCAGAGTGTTAGCAGCAGCTACTTAGCCTGGTACCAGCAGAAACCTGGCCAGGCTCCAG
 GCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGGCATCCAGACAGGTTCAAGTGGCAGT
 GGGTCTGGGACAGACTTCACTCTCACCATCAGCAGACTGGAGCCTGAAGATTTTGCAGTGT
 ATTACTGTCAGCAGTATGGTAGCTCACCTCCCACAGTGATTCAGCTTGAAACAAAAACCTCT
 GCAAGACCTTCATTGTTTACTAGAT TATACCAGCTG (Seq ID NO 155)

JK

L3

-

CDR3

--

100

|

Jk1 WTFGQGTKVEIK (Seq ID NO. 156)

Figure 24

pAXB116 Fab'-gVh

(SEQ ID NO 157) ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCT GCC CAA
 (SEQ ID NO 158) M K Y L L P T A A A G L L L L A A Q
pelB leader
 CCA GCC ATG GCG CAG GTG CAG CTG GTG CAG AGC GGC GCG GAA GTG AAA AAA CCG
 P A M A Q V Q L V Q S G A E V K K P pAXB116 Fab'-gVh
 (SEQ ID NO 159) Q S G A E V K K P TT-Vh(CDR3-TPO)
 (SEQ ID NO 169) Q V Q L V Q S G A E V K K P L22582

CDR1-H1
 GGC AGC AGC GTG AAA GTG AGC TGC AAA GCG AGC GGC GGC ACC TTT AGC AGC TAT
 G S S V K V S C K A S G G T F S S Y pAXB116 Fab'-gVh
 G S S V K V S C R A S G G T F N N Y TT-Vh(CDR3-TPO)
 G S S V K V S C K A S G G T F S S Y L22582

GCG ATT AGC TGG GTG CGC CAG GCG CCG GGC CAG GGC CTG GAA TGG ATG GGC CAG
 A I S W V R Q A P G Q G L E W M G Q pAXB116 Fab'-gVh
 A I S W V R Q A P G Q G L E W M G G TT-Vh(CDR3-TPO)
 A I S W V R Q A P G Q G L E W M G G L22582

CDR2-TPO
CTG ATT GAA GGC CCG ACC CTG CGC CAG TGG CTG GCG GCG GCG GCG AAC AGC CGC
 L I E G P T L R Q W L A A R A N S R pAXB116 Fab'-gVh
 I F P F R N T A K Y A Q H F Q G R TT-Vh(CDR3-TPO)
 I I P I F G T A N Y A Q K F Q G R L22582

GTG ACC ATT ACC GCG GAT GAA AGC ACC AGC ACC GCG TAT ATG GAA CTG AGC AGC
 V T I T A D E S T S T A Y M E L S S pAXB116 Fab'-gVh
 V T I T A D E S T G T A Y M E L S S TT-Vh(CDR3-TPO)
 V T I T A D E S T S T A Y M E L S S L22582

CTG CGC AGC GAA GAT ACC GCG GTG TAT TAT TGC GCG CGC CTG CCG ATT GAA GGC
 L R S E D T A V Y Y C A R L P I E G pAXB116 Fab'-gVh
 L R S E D T A I Y Y C A R L P I E G TT-Vh(CDR3-TPO)
 L R S E D T A V Y Y C A R L22582

CDR3-TPO
CCG ACC CTG CGC CAG TGG CTG GCG GCG GCG GCG CCG GTG TGG GGC CAG GGC ACC
 P T L R Q W L A A R A P V W G Q G T pAXB116 Fab'-gVh
 P T L R Q W L A A R A P V W G Q G T TT-Vh(CDR3-TPO)

ACC GTG ACC GTG AGC AGC
 T V T V S S pAXB116
 Fab'-gVh
 T V T V S A TT-Vh(CDR3-TPO)

Fig 24 Sequence of the pAXB116 Fab' Heavy chain variable region. The cDNA sequence with the best E. coli codon usage (Henaut and Danchin, 1996) and the translated amino acid sequence of pAXB116 Fab' are shown. CDR (Complementarity Determining Region) are defined by Kabat et al(1992) and the structural variability definition sequence (Chothia and Lesk, 1987) underlined and overlined, respectively. TPO peptides in heavy chain CDR2 and CDR3 of pAXB116 Fab' are indicated by double underlines and wavelines respectively. pelB leader cDNA sequences are overlined. pAXB116 Fab'-gVh denotes heavy chain variable region of human germline derived pAXB116 Fab'.

Figure 25

pAXB116 Fab' gV_k

		<u>pelB leader</u>	
(SEQ ID NO. 160)	ATG AAA TAC CTA TTG CCT ACG GCA GCC GCT GGA TTG TTA TTA CTC GCT GCC CAA		
(SEQ ID NO. 161)	M K Y L L P T A A A G L L L L A A Q		
		<u>pelB leader</u>	
		<u>CCA GCC ATG GCG GAA ATT GTG CTG ACC CAG AGC CCG GGC ACC CTG AGC CTG AGC</u>	
		P A M A E I V L T Q S P G T L S L S	
		(SEQ ID NO. 162) E L T Q S P G T L S L S	
		(SEQ ID NO. 170) E I V L T Q S P G T L S L S	
		pAXB116 Fab'-gV _k	
		TTV _k	
		X12686	
		<u>CDR1-L1</u>	
		<u>CCG GGC GAA CGC GCG ACC CTG AGC TGC CGC GCG AGC CAG AGC GTG AGC AGC AGC</u>	
		P G E R A T L S C R A S Q S V S S S	
		P G E R A T L S C R A S H S V S R A	
		P G E R A T L S C R A S Q S V S S S	
		pAXB116 Fab'-gV _k	
		TTV _k	
		X12686	
		<u>TAT CTG GCG TGG TAT CAG CAG AAA CCG GGC CAG GCG CCG CGC CTG CTG ATT TAT</u>	
		Y L A W Y Q Q K P G Q A P R L L I Y	
		Y L A W Y Q Q K P G Q A P R L L I Y	
		Y L A W Y Q Q K P G Q A P R L L I Y	
		pAXB116 Fab'-gV _k	
		TTV _k	
		X12686	
		<u>CDR2-L2</u>	
		<u>GGC GCG AGC AGC CGC GCG ACC GGC ATT CCG GAT CGC TTT AGC GGC AGC GGC AGC</u>	
		G A S S R A T G I P D R F S G S G S	
		G T S S R A T G I P D R F S G S G S	
		G A S S R A T G I P D R F S G S G S	
		pAXB116 Fab'-gV _k	
		TTV _k	
		X12686	
		<u>GGC ACC GAT TTT ACC CTG ACC ATT AGC CGC CTG GAA CCG GAA GAT TTT GCG GTG</u>	
		G T D F T L T I S R L E P E D F A V	
		G T D F T L T I S R L E P E D F A V	
		G T D F T L T I S R L E P E D F A V	
		pAXB116 Fab'-gV _k	
		TTV _k	
		X12686	
		<u>CDR3-L3</u>	
		<u>TAT TAT TGC CAG CAG TAT GGC AGC AGC CCG TGG ACC TTT GGC CAG GGC ACC AAA</u>	
		Y Y C Q Q Y G S S P W T F G Q G T K	
		Y Y C Q Q Y G G S P W F G Q G T K	
		Y Y C Q Q Y G S S P F G Q G T K	
		pAXB116 Fab'-gV _k	
		TTV _k	
		X12686	
		GTG GAA ATT AAA	
		V E I K	
		Fab'-gV _k	
		V E L K	
		pAXB116	
		TTV _k	

Fig 25 Sequence of the pAXB116 Fab' Light chain variable region. The cDNA sequence with the best E. coli codon usage (Henaut and Danchin, 1996) and the translated amino acid sequence of pAXB116 Fab' are shown. CDR (Complementarity Determining Region) are defined by Kabat et al(1992) and the structural variability definition sequence (Chothia and Lesk, 1987) underlined and overlined, respectively. pelB leader cDNA sequences are overlined. pAXB116 Fab'-gV_k denotes light chain variable region of human germline derived pAXB116 Fab'.

Figure 26 - Primers to generate pAXB116 heavy chain

UDEC1709 : 5' primer 272 bp, containing NcoI site (seq ID No. 163)

5'—CCAGCCATGCGCGCAGGTGCAGCTGGTGCAGAGCGGCGCGGAAGTGAAAAAACCGGGCAGCAGCGTGAAAGT
GAGCTGCAAAAGCGAGCGGCGGCACCTTTAGCAGCTATGCGATTAGCTGGGTGCGCCAGCGCGCGGGCCAGGGCCTG
GAATGGATGGCGGCATTATTCGATTCTTGGCACCGCGAACTATGCGCAGAAATTCAGGGCGCGGTGACCATTA
CGCGGATGAAAGCACCAGCACCGCGTATATGGAACAGCAGCCTGCG—3'

Overlapping with UDEC1710

UDEC1710 3' primer 271 bp

5'—GTTCCAGCTCACGGTCAACGGTTCCGGAAAAATAATCTTTACACAGGCAGCCAGCGCCGCGGTGCCGCCG
Overlapping with UDEC1711

CTGGTGCTTTTGTGCTCGGCGCCAGCGGAAACACGCTCGGGCCTTTGGTGCTCGCGCTGCTCACGGTCAACGGTGGT
GCCCTGGCCCCACACCGGCGCGCGCGCCAGCCACTGGCGCAGGCTCGGGCCTTCAATCGGCAGGCGCGCGCAA
TAATACACCGCGGTATCTTCGTGCGCAGGCTGCTCAGTTCCATATAC—3' (seq ID No. 164)

Overlapping with UDEC1709

UDEC1711 3' primer (274 bp) containing XbaI site

5'—CGAGTCTAGATTACGGGCGCGCCAGCAATTCCGGCGCGGGCAGCGCGGGCAGGTATGGGTTTATCGCAGCT
TTTCGGTTCCACTTTTTATCCACTTTGGTGTGCTCGGTTTATGGTTACGTTGCAAAATATAGGTCTGGGTGCCAGG
CTGCTGCTCGGCACGGTCAACCGCTGCTCAGGCTATACAGCGCGCTGCTCTGCAGCACCGCGGAAAGGTATGCAC
GCCGCTGGTCAGCGCGCGCTGTTCCAGCTCACGGTCAACGGTTC—3' (seq ID No. 165)

overlapping with UDEC1710

Figure 27 - Primers to generate pAXB116 -light chain

UDEC1712 5' primer 236 bp

5'—CCAGCCATGGCGGAAATTGTGCTGACCCAGAGCCCGGGCACCTGAGCCTGAGCCCGGGCGAACCGCGGAC
CCTGAGCTGCCGCGCGAGCCAGAGCGTGAGCAGCAGCTATCTGGCGTGGTATCAGCAGAAACCGGGCCAGGCGCGG
CGCTGCTGATTTATGGCGGAGCAGCGCGCGACCGGCAATCCGGATCGCTTAGCGGCAGCGGCAAGCGGACCG
ATTTTACCTGAC—3' (seq ID No. 166)

Overlapping with udec1713 (24bp)

UDEC1713 3' primer 239bp

5'—CTTTCGCTTCGCGCGGATAAAAGTTGTTTCAGCAGGCACACCAGCTCGCGGTGCCGCTTTTCAGTGTTCA

overlapping with UDEC1714

TEGCTCGGCGGAAAAATAAACACGCTCGCGCGCGCACGGTGCCTTTAATTTCCACTTTGGTGCCCTGGCCAAAGGT
CCACGGGCTGCTGCCATACTGCTGGCAATAATACACCGCAAAATCTTCGGTTCCAGGCGGCTAATGGTCAGGGTAA
AATCGGTGCCGCTG—3' (seq ID No. 167)

Overlapping with udec1712(24bp)

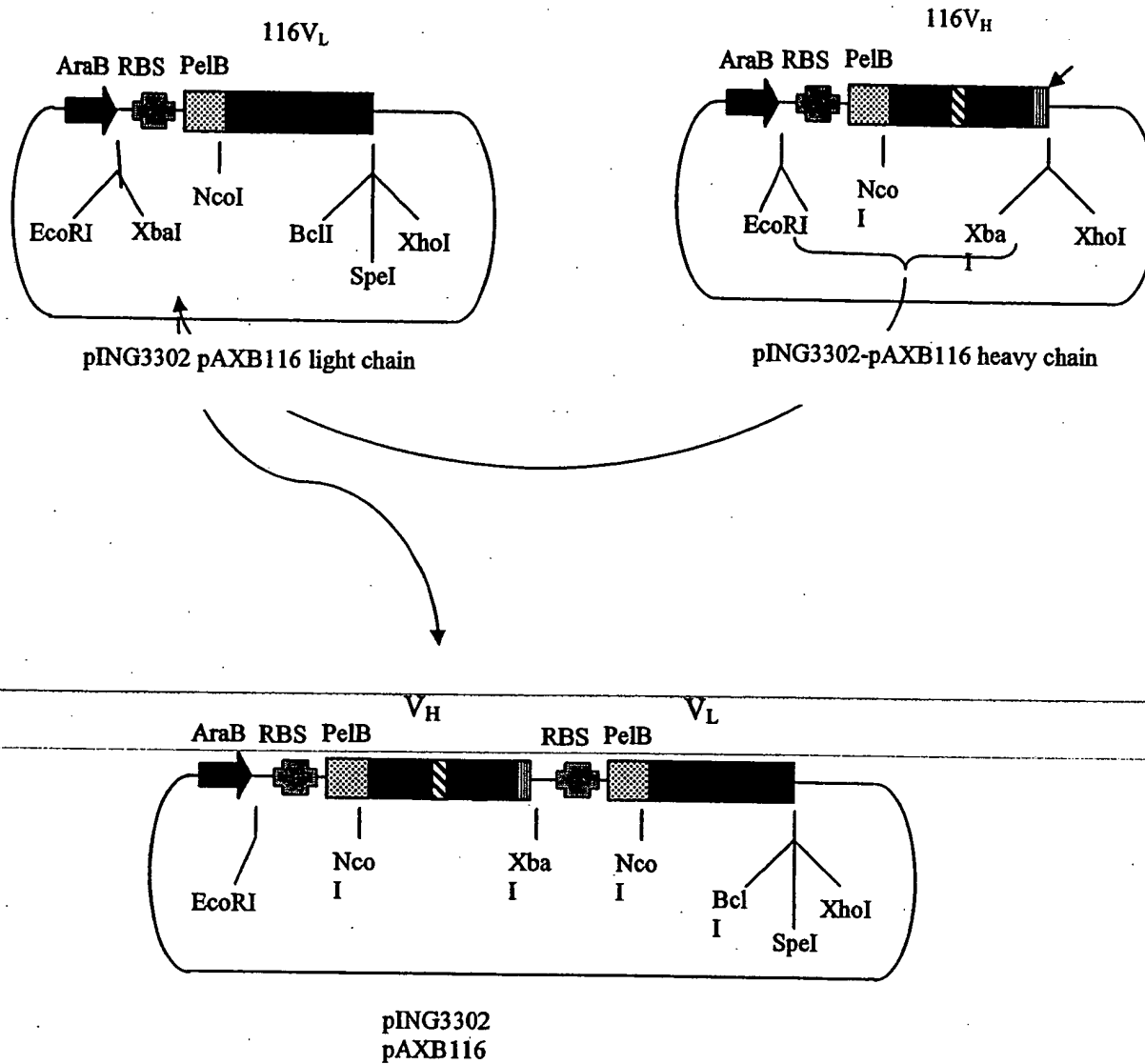
UDEC1714 3' primer 245 bp

5'—GTGCTGATCATTAGCATTGCGCGGTTAAAGCTTTTGGTCACCGGCAGGCTCAGGCCCTGATGGGTCACTTC
GCACGCATACACTTTATGTTTTTCATAATCCGCTTTGCTCAGGGTCAGGGTGCTGCTCAGGCTATAGGTGCTATCTTT
GCTATCCTGTTTCGGTCAACGCTTTCCTGGCTGTTGCCGCTTGCAGCGGTTATCCACTTCCACTGCACTTTCGCTTCG
CGCGGATAAAAGTTG—3' (seq ID No. 168)

overlapping with udec1713 (26 bp)

Figure 28

Construction scheme for pING-pAXB116



116 Light Chain (SEQ. ID NO. 122):

EIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSG
SGSGTDFLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPRKAKVQWKVDNALQSGNSQESVTEQDSKSTYSLSSLTLSKADYEKHKVYA
CEVTHQGLSLPVTKSFNREGC.

Variable Region of 116 Light Chain (SEQ. ID NO. 123):

EIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSG
SGSGTDFLTISRLEPEDFAVYYCQQYGSSPWTFGQGTKVEIK

116 Heavy Chain (SEQ. ID NO. 124):

QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVRQAPGQGLEWMGQLIEGPTLRQWLA
ARANSRVITADESTSTAYMELSSLRSEDTAVYYCARLPIEGPTLRQWLAARAPVWGQGTITV
SSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKK { **VEPKSCDKTHTCPPCP** } *APELLGGP*

end CH1 constant domain

hinge region

tail region

Variable Region of 116 Heavy Chain (SEQ. ID NO. 125):

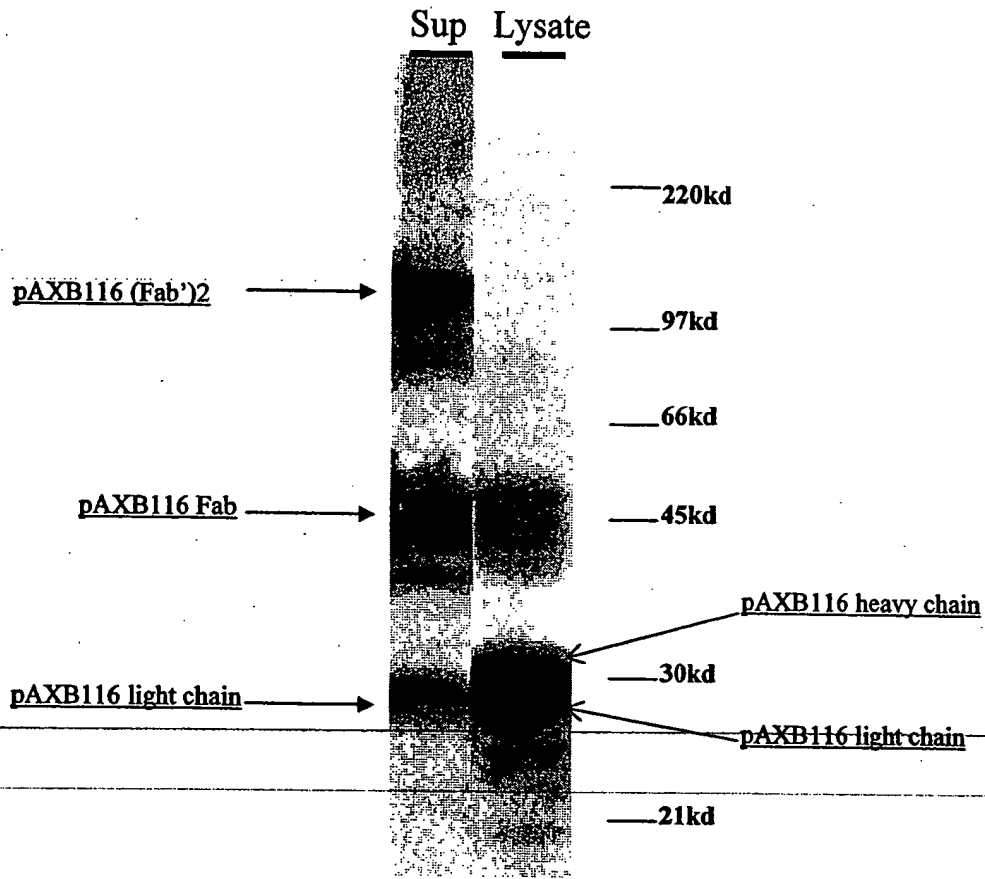
QVQLVQSGAEVKKPGSSVKVSCASGGTFSSYAISWVRQAPGQGLEWMGQLIEGPTLRQWLA
ARANSRVITADESTSTAYMELSSLRSEDTAVYYCARLPIEGPTLRQWLAARAPVWGQGTITV
SS

Clone 116. The light chain sequence is as given. The heavy chain may have several forms depending on the final antibody form. For example: the minimum sequence ending with the CH1 constant domain may result in Fab assembly but the heavy chain/light chain interaction will not be stable. Commonly, a portion of the hinge region containing a cysteine (the underlined bold portion of hinge region) may be included for a covalent interaction between the heavy and light chains. Fab₂ association would need further cysteines such as an entire IgG₁ hinge region (bold). In this example, clone 116 was cloned in a Xoma pING3302 modified vector which includes a transition tail region (italicized).

Fig. 29

Figure 30

SDS-PAGE of pAXB116, culture supernatant was resolved from each other by non-reducing 4-12% SDS-PAGE (lane "Sup") and cell lysate by reducing 4-12% SDS-PAGE (lane "Lysate"). Proteins were transferred onto Hybond Nitrocellulose Sheet (Amersham) and block with TBS-0.2% Tween-20+10% (w/v) Carnation nonfat dry milk. PAXb116 were detected by HRP-conjugated goat-antiHuman (H+L)Ab (Chenicon Cat#AP112P, (Temecula, CA). The signals were detected by ECL.



WB:Goat anti human(H+L) HRP-I:1000

Fig. 31

Proliferative Effect of TPO on CD 34+ Cord Blood Cells

CD34⁺ cord blood cells (Poeisis) were thawed, washed, resuspended in BIT9500 serum-substituted medium (StemCell Technologies, Inc.), and plated at 3.5×10^5 per well in a 96 well flat-bottom plate with increasing concentrations of either recombinant human TPO (R&D Systems), circle, or pAXB116, square. After four days of culture at 37°C in a 5% CO₂ incubator, 1 μ Ci of ³H thymidine (Perkin Elmer) was added to each well and cells were further incubated for 16 hours. Cells were harvested with an automatic 96-well cell harvester. ³H incorporation was measured using a betaplate liquid scintillation counter (Wallac). Proliferation of cord blood cells are measured as counts per minute (CPM) and CPM values are an average of three wells.

Proliferative Effect of TPO and 116 on
CD 34+ Cord Blood Cells

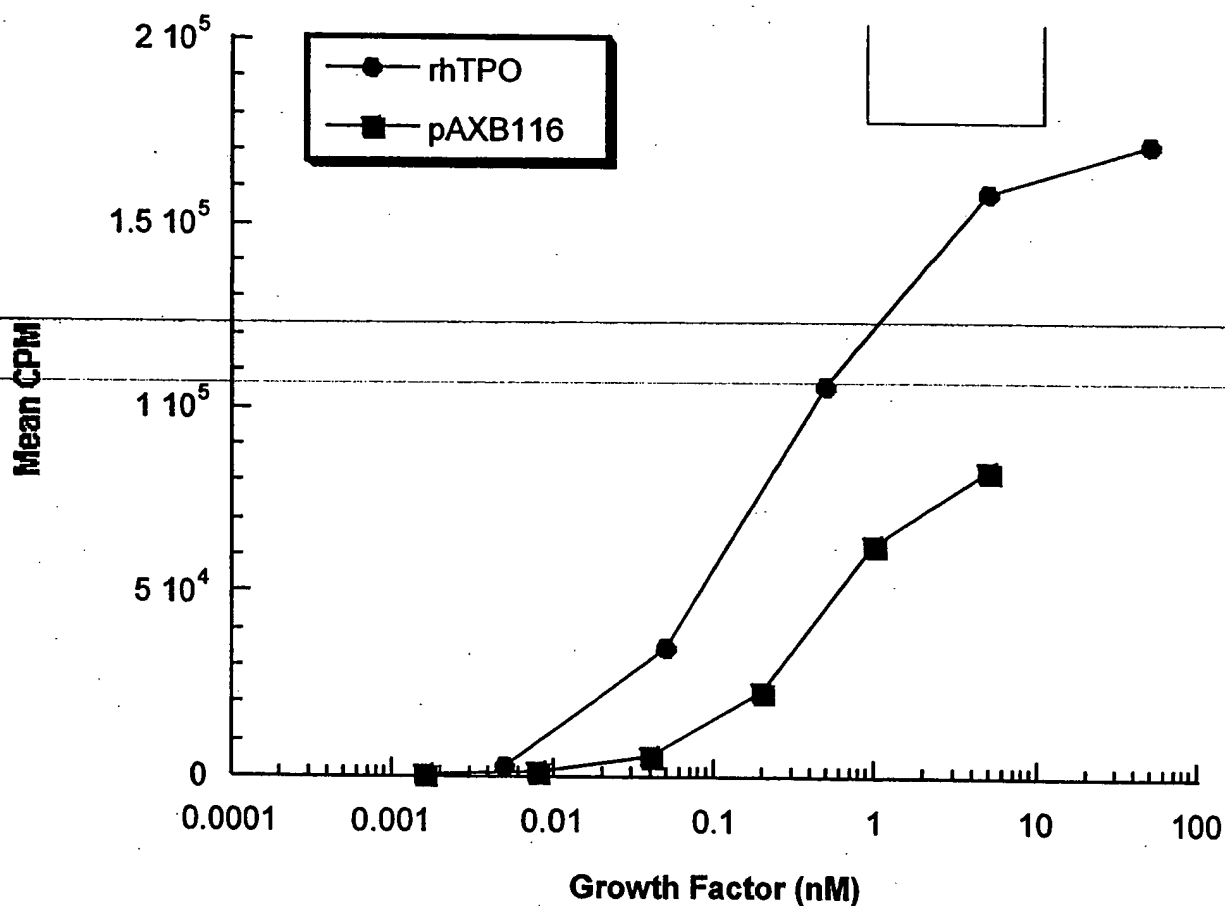
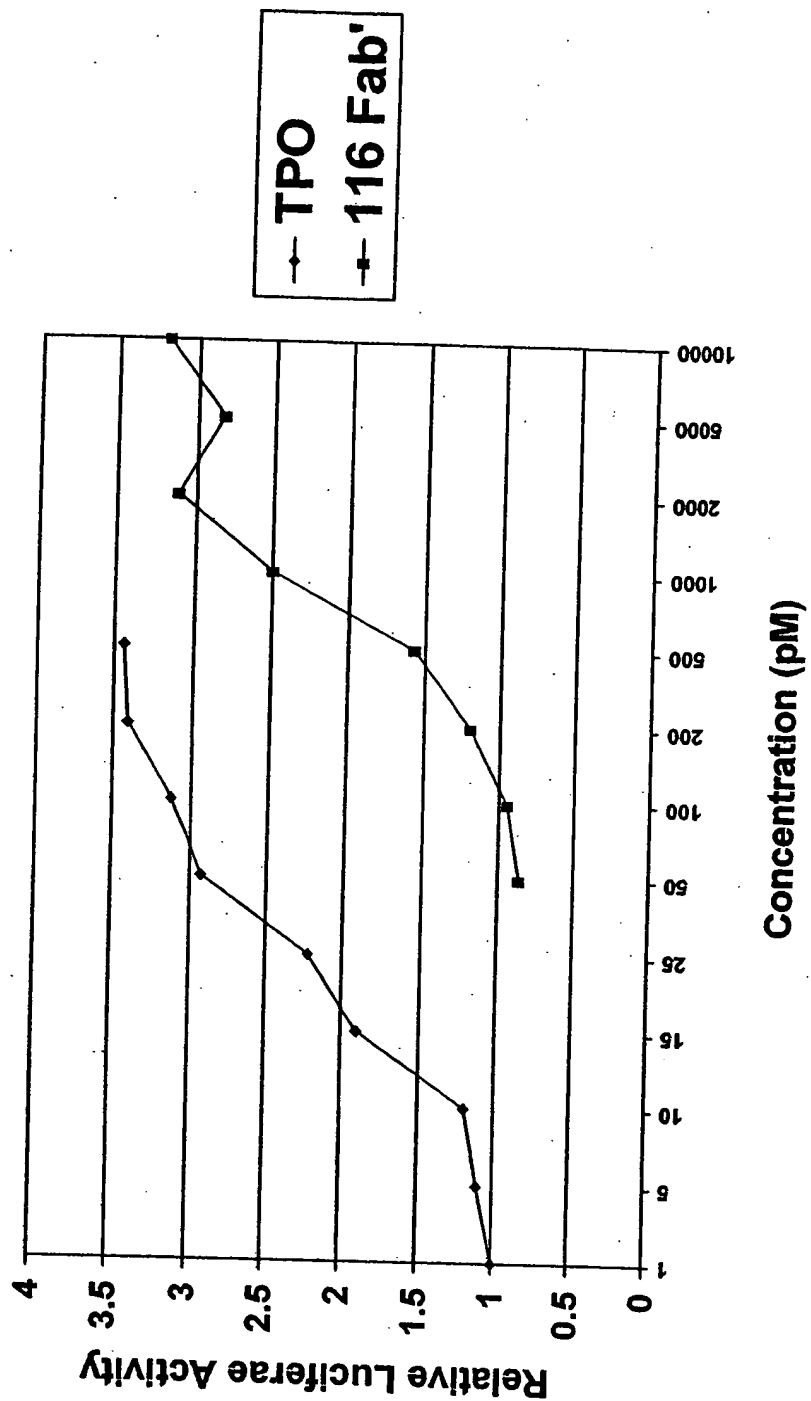


Figure 32: Activity of 116 Fab' prep #5



Sample Sequences of Heavy Chain CDR2 clones

Gly-Ile-Phe-xxx-xxx-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-xxx-xxx-Gly (SEQ. ID NO. 126)

TT backbone randomized TPO peptide randomized TT backbone

Amino Acid Sequence

HR2-14 Gly-Ile-Phe-Ser-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Gly-Gly (SEQ. ID NO. 127)

HR2-20 Gly-Ile-Phe-Pro-Gln-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Lys-Gly (SEQ. ID NO. 128)

HR2-23 Gly-Ile-Phe-Pro-Asn-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Thr-Gly-Gly (SEQ. ID NO. 129)

HR2-28 Gly-Ile-Phe-Lys-Gly-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Pro-Gly-Gly (SEQ. ID NO. 130)

HR2-43 Gly-Ile-Phe-Pro-Pro-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Val-Gly (SEQ. ID NO. 131)

HR2-44 Gly-Ile-Phe-Pro-Arg-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Lys-Leu-Gly (SEQ. ID NO. 132)

IR2-48 Gly-Ile-Phe-Pro-Arg-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Arg-Ala-Lys-Leu-Gly (SEQ. ID NO. 133)

HR2-50 Gly-Ile-Phe-Pro-Tyr-Ile-Glu-Gly-Pro-Thr-Leu-Arg-Gln-Trp-Leu-Ala-Ala-Arg-Ala-Lys-Arg-Gly (SEQ. ID NO. 134)

Figure 34 Relative Activity of 2° H2/H3-(X4b) clones

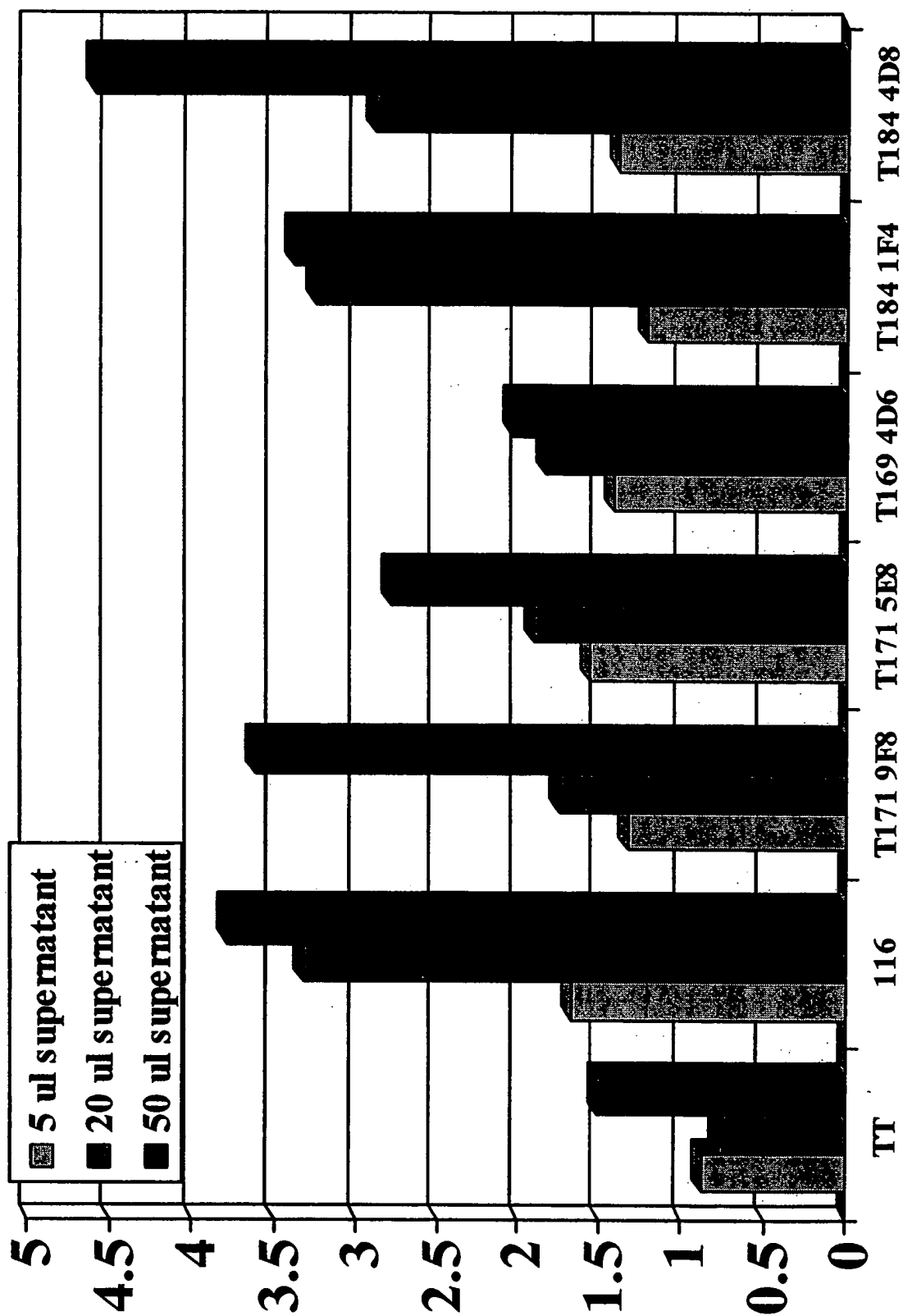


Figure 35: Optimization of the TPO placement in HC-CDR2

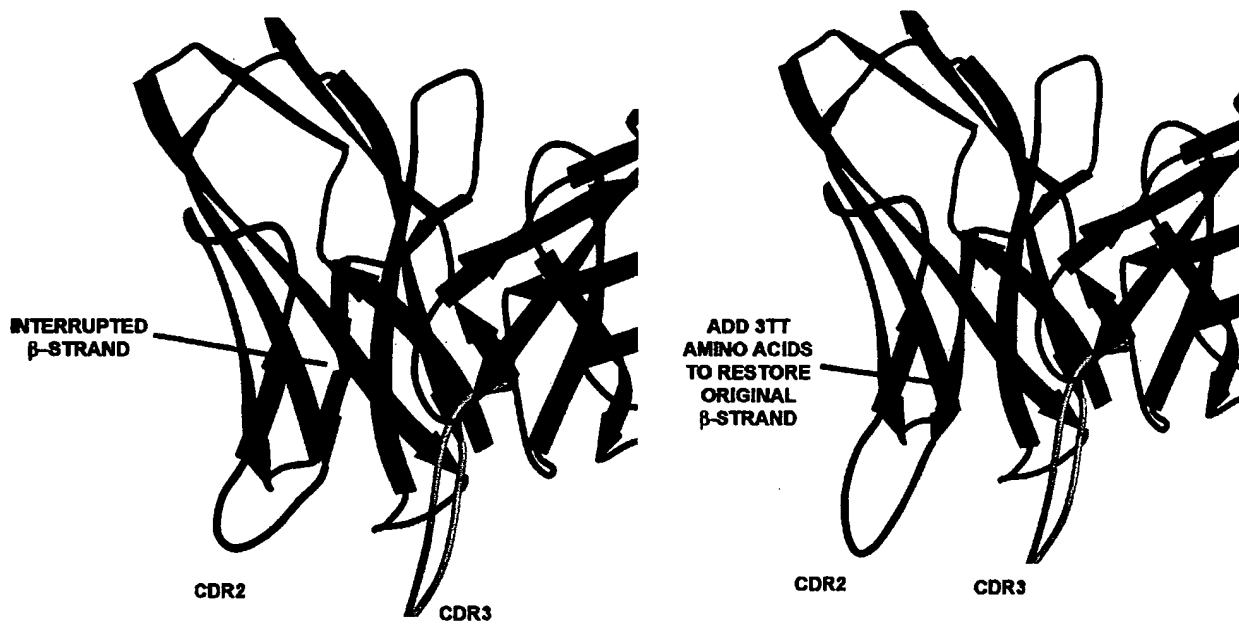


Figure 36A (SEQ. ID. NO. 141)

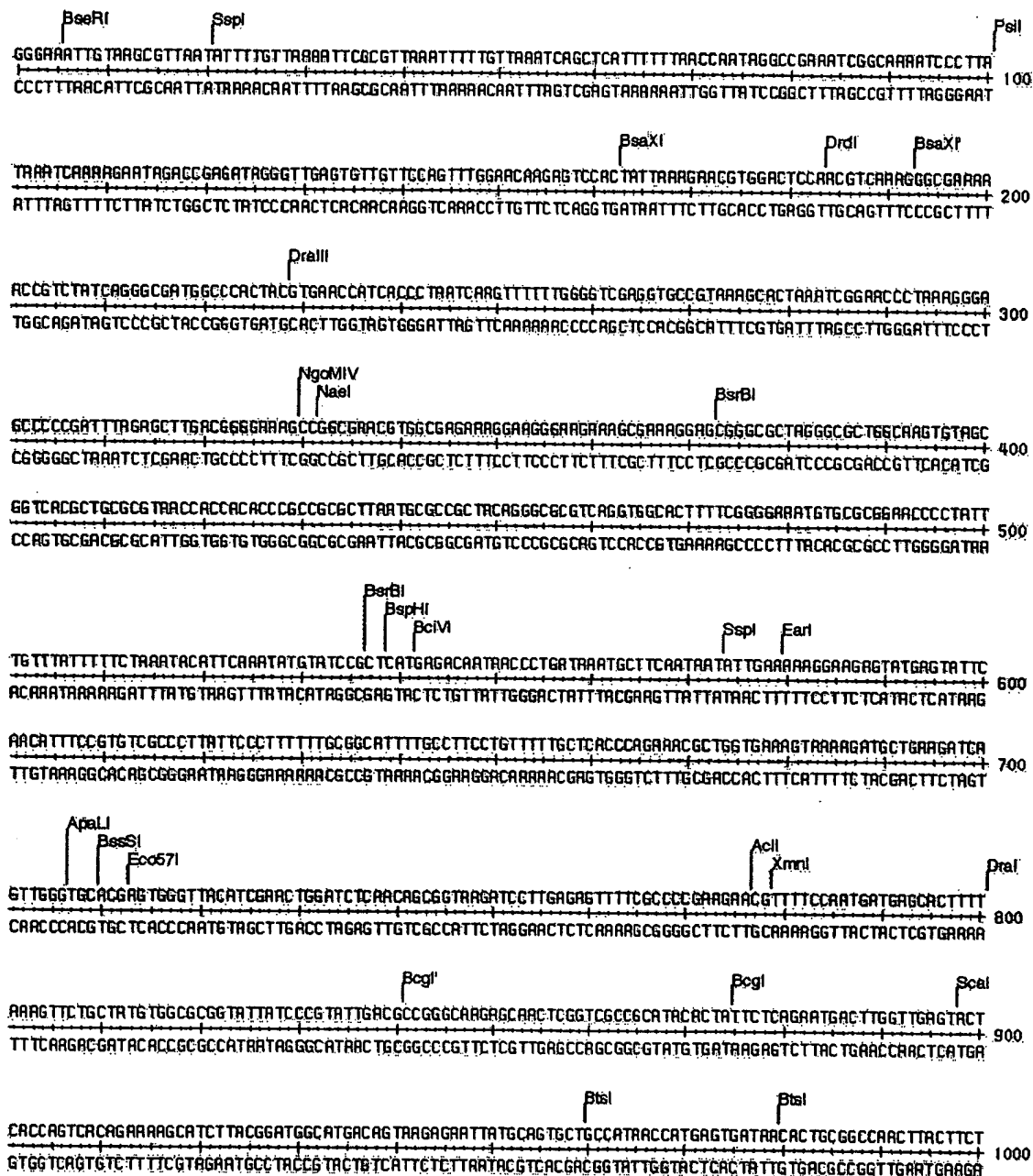


Figure 36B

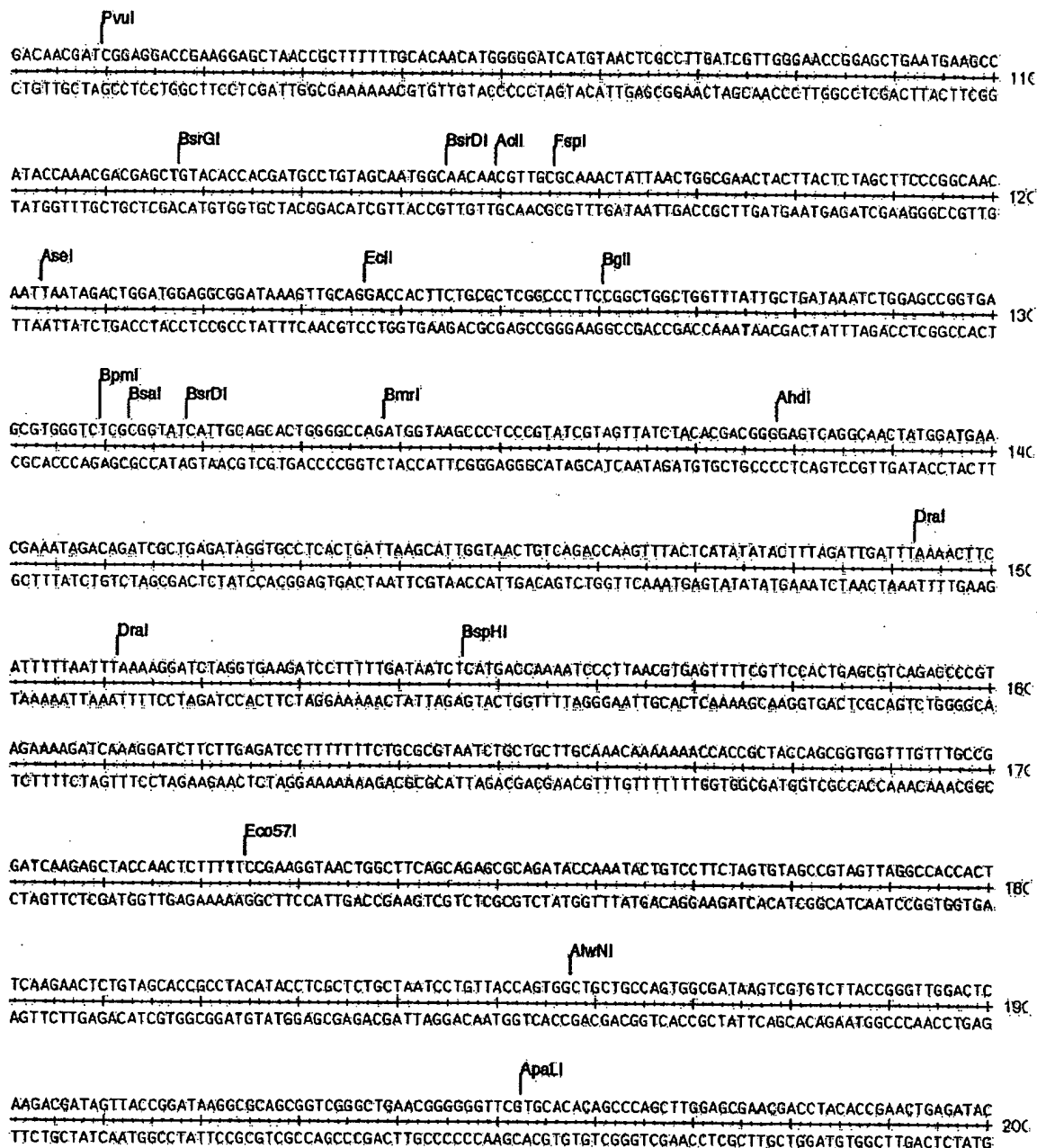
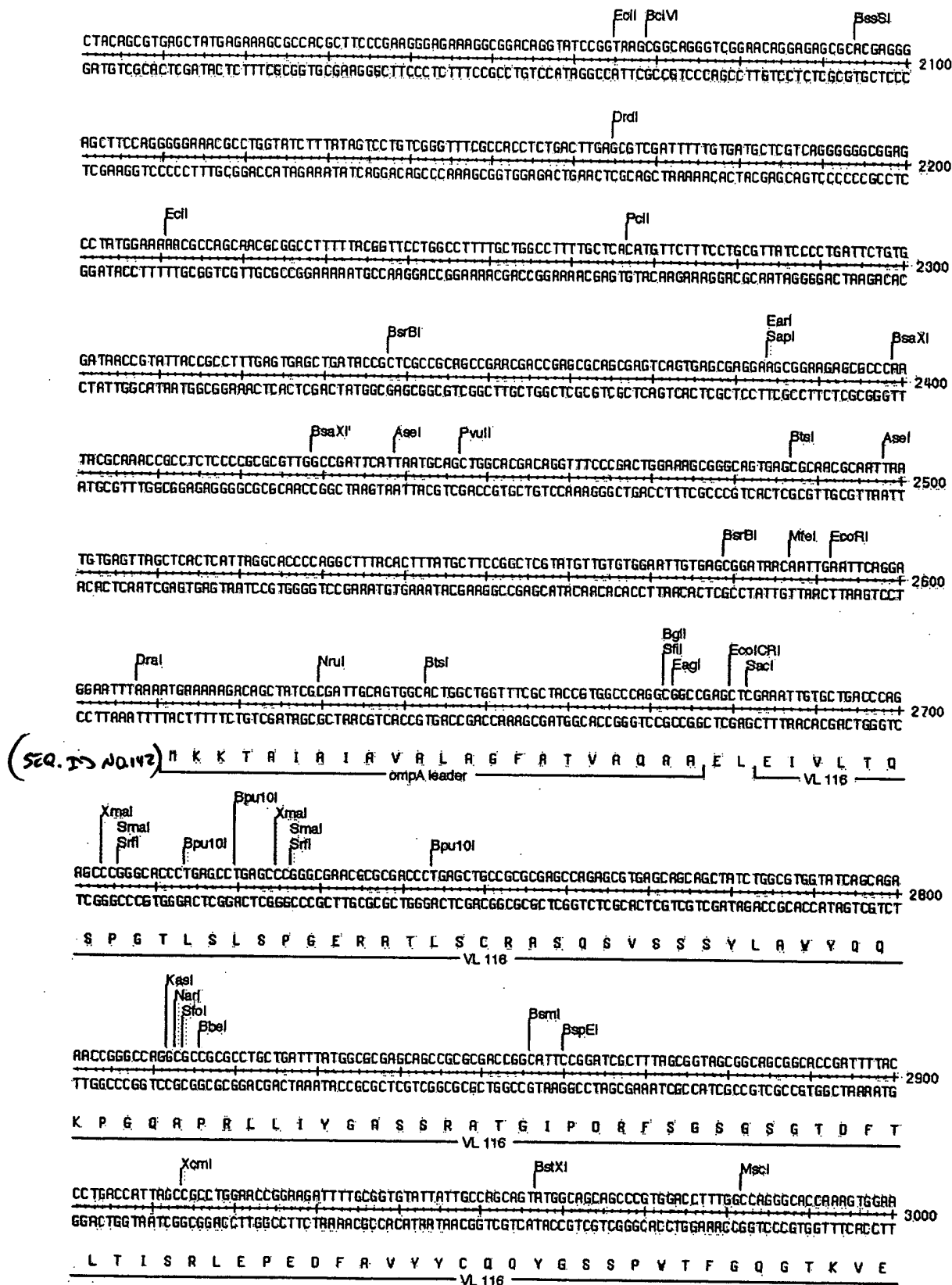


Figure 36C



50D

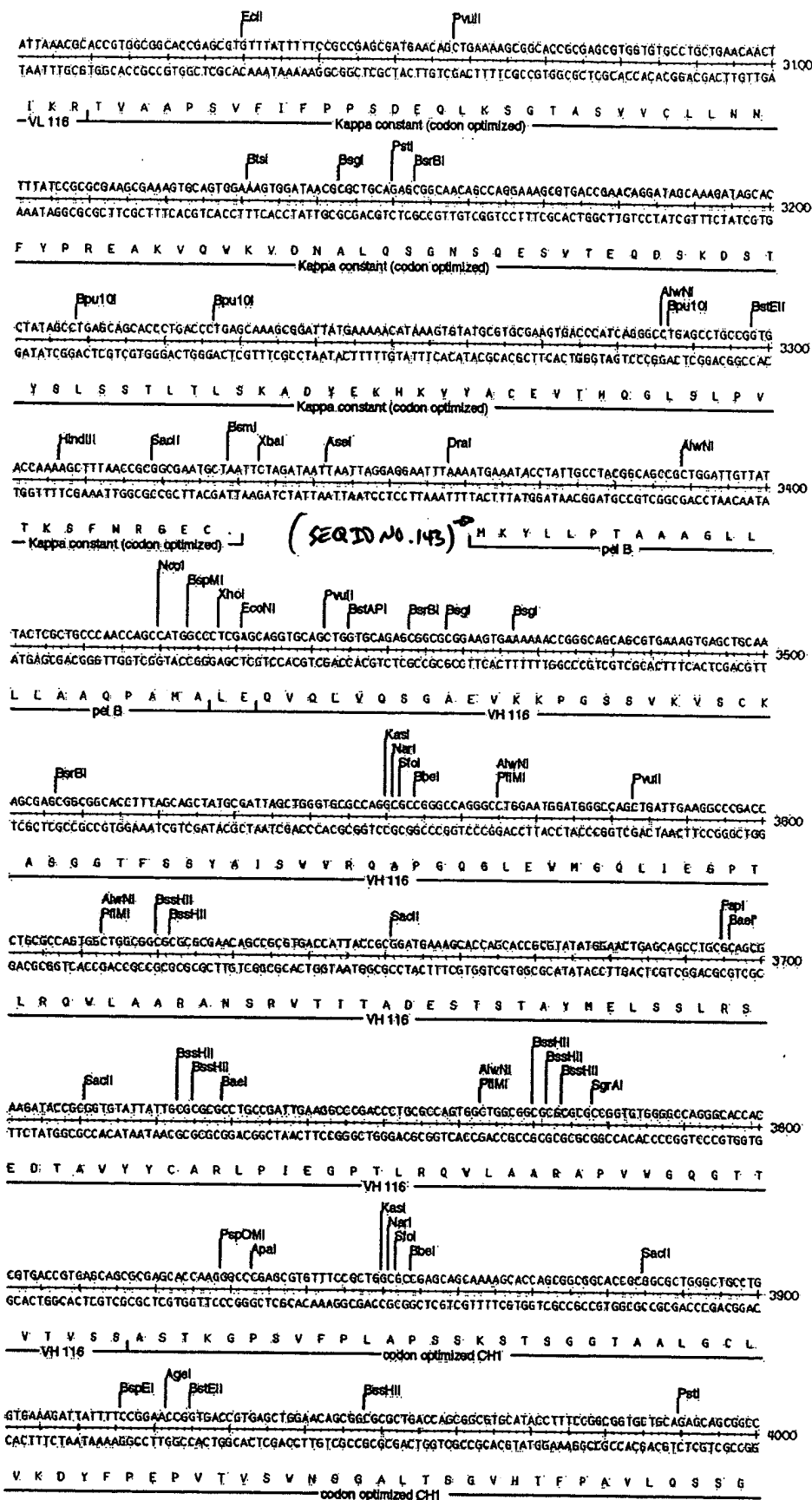


Figure 36E

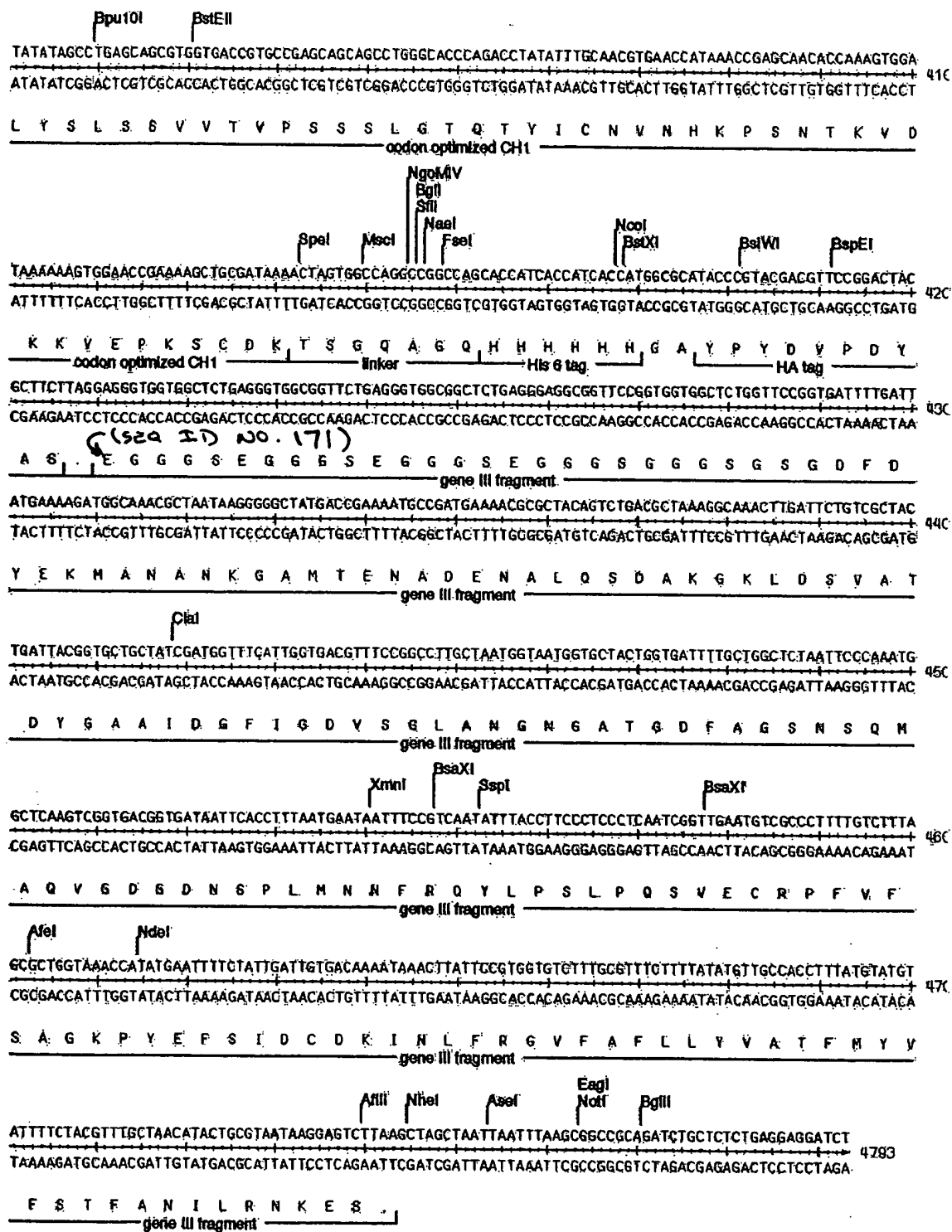


Figure 37 Relative Activity of 116 Mutants

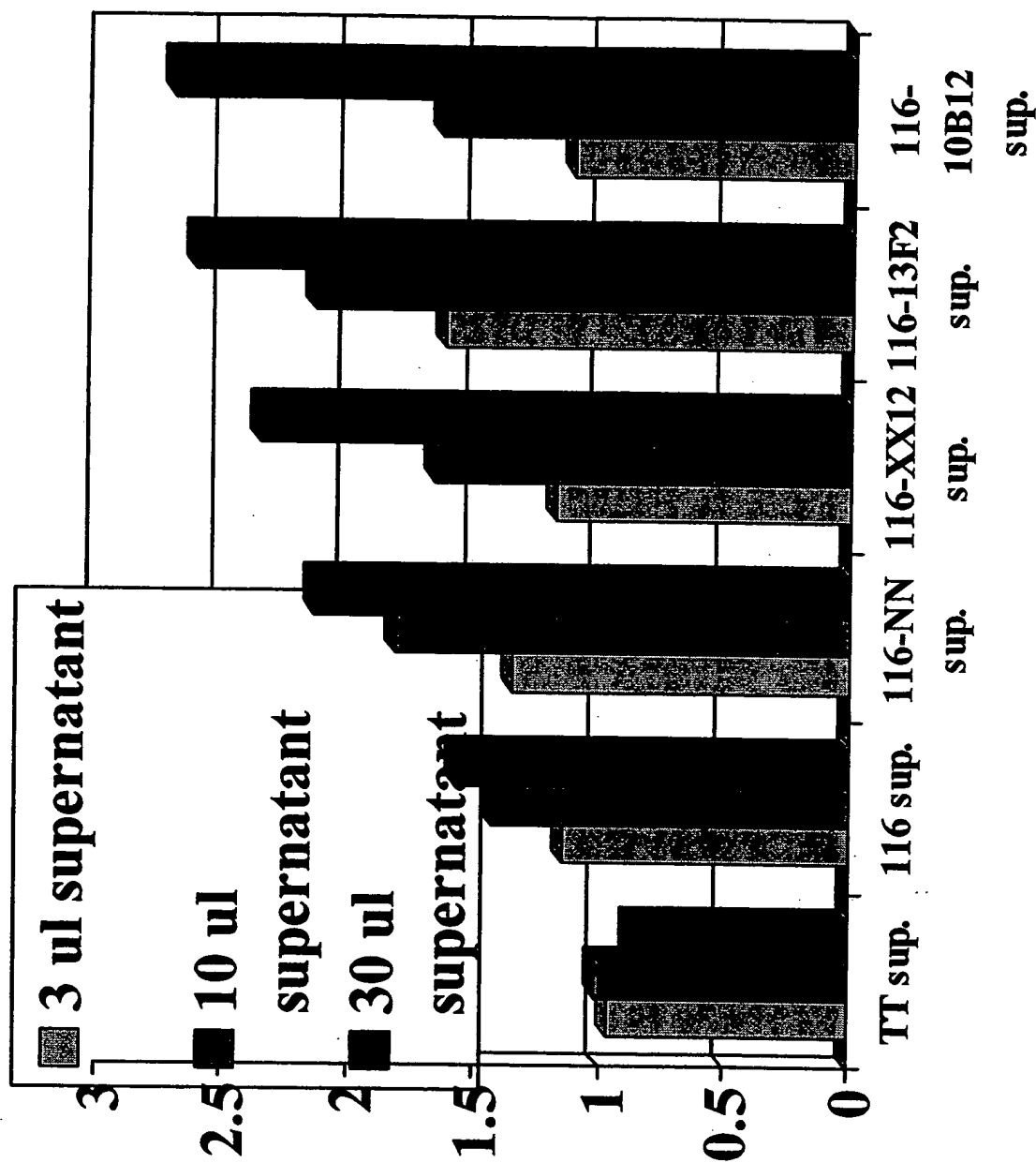


Figure 38 116 Variants Alignment

		10	20	30	
1	Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F S				pRL5-116 (VH)
1	Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F N				pRL5-116 NN (VH)
1	Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F G				pRL5-116 10B12 (VH)
1	Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F Q				pRL5-116 13F2 (VH)
1	Q V Q L V Q S G A E V K K P G S S V K V S C K A S G G T F P				pRL5-116 XX12 (VH)

		40	50	60	
31	S Y A I S W V R Q A P G Q G L E W M G Q L I E G P T L R Q W				pRL5-116 (VH)
31	N Y A I S W V R Q A P G Q G L E W M G Q L I E G P T L R Q W				pRL5-116 NN (VH)
31	E Y A I S W V R Q A P G Q G L E W M G Q L I E G P T L R Q W				pRL5-116 10B12 (VH)
31	D Y A I S W V R Q A P G Q G L E W M G Q L I E G P T L R Q W				pRL5-116 13F2 (VH)
31	R Y A I S W V R Q A P G Q G L E W M G Q L I E G P T L R Q W				pRL5-116 XX12 (VH)

		70	80	90	
61	L A A R A N S R V T I T A D E S T S T A Y M E L S S L R S E				pRL5-116 (VH)
61	L A A R A N S R V T I T A D E S T S T A Y M E L S S L R S E				pRL5-116 NN (VH)
61	L A A R A N S R V T I T A D E S T S T A Y M E L S S L R S E				pRL5-116 10B12 (VH)
61	L A A R A N S R V T I T A D E S T S T A Y M E L S S L R S E				pRL5-116 13F2 (VH)
61	L A A R A N S R V T I T A D E S T S T A Y M E L S S L R S E				pRL5-116 XX12 (VH)

		100	110	120	
91	D T A V Y Y C A R L P I E G P T L R Q W L A A R A P V W G Q				pRL5-116 (VH)
91	D T A V Y Y C A R L P I E G P T L R Q W L A A R A P V W G Q				pRL5-116 NN (VH)
91	D T A V Y Y C A R L P I E G P T L R Q W L A A R A P V W G Q				pRL5-116 10B12 (VH)
91	D T A V Y Y C A R L P I E G P T L R Q W L A A R A P V W G Q				pRL5-116 13F2 (VH)
91	D T A V Y Y C A R L P I E G P T L R Q W L A A R A P V W G Q				pRL5-116 XX12 (VH)

121	G T T V T V S S	(SEQ. ID No. 147)	pRL5-116 (VH)
121	G T T V T V S S	(SEQ. ID No. 148)	pRL5-116 NN (VH)
121	G T T V T V S S	(SEQ. ID No. 149)	pRL5-116 10B12 (VH)
121	G T T V T V S S	(SEQ. ID No. 150)	pRL5-116 13F2 (VH)
121	G T T V T V S S	(SEQ. ID No. 151)	pRL5-116 XX12 (VH)